

The genital disc of *Drosophila melanogaster*: a developmental and genetic analysis of its sexual dimorphism

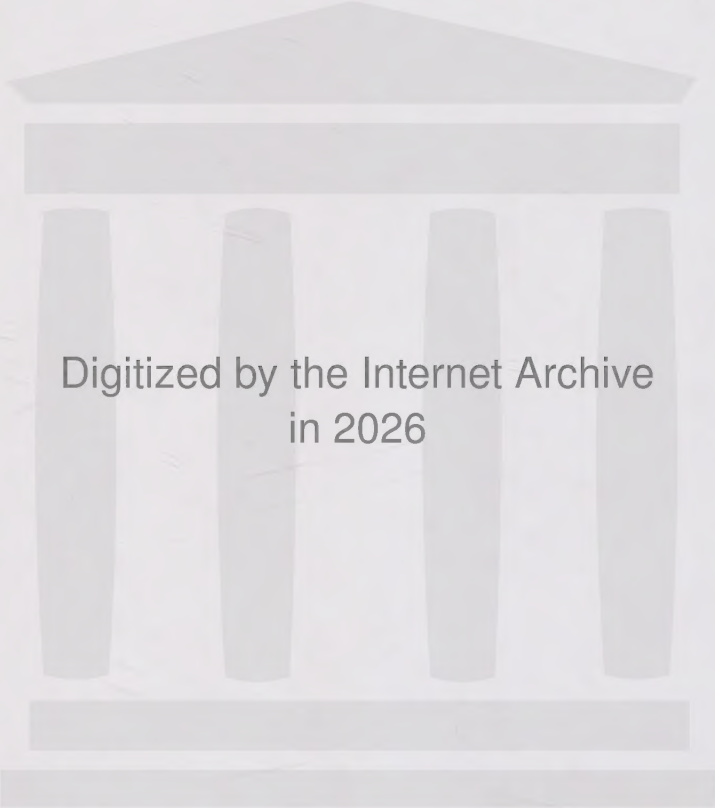
Inaugural-Dissertation

zur Erlangung der philosophischen Doktorwürde
vorgelegt der
Philosophischen Fakultät II
der
Universität Zürich

von
Felix Epper-Keller
von Bischofszell/TG
und Heldswil/TG

Begutachtet von Prof. Dr. R. Nöthiger, Zürich
und Prof. Dr. W. Janning, Münster (Westfalen)

Zürich 1980
W. Schneider, Druckerei, Zürich



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S U M M A R Y

This study reports on the sexually dimorphic organization of the genital imaginal disc which gives rise to the terminalia (genitalia and analia) of Drosophila melanogaster. At blastoderm stage, the embryonic anlage of the genital disc contains three terminal primordia: one for the analia, one for the male and one for the female genitalia. Since the terminalia of the adult fly comprise only elements of one genital primordium, the development of the genital primordium of opposite sex must be "repressed".

The aim of this study was

- i) to localize the "repressed" genital primordium in the genital disc of male and female animals
- ii) to study the organization of the genital disc in animals with an intersexual genotype that gives rise to male and female genital elements.

In order to find the "repressed" male genital primordium (RMP) in the female disc, I tried to reveal the site in the disc from where the male genital clones originated.

For this purpose, clones were induced by irradiating M(3)i⁵⁵/tra female larvae with 2000 rads towards the end of the third instar. The discs were isolated from mature larvae and cultured for 10 - 14 d in adult females to allow for extensive growth of the male tra/tra clones.

After the in vivo culture, 19 of 224 cultured discs showed clearly noticeable growth in the anterior third of the dorsal disc epithelium (Figure 16a). This tissue was cut out of the disc and in 11 cases successfully transplanted into

larval hosts. In each case the tissue differentiated male genital structures (Figure 16b, c, Table 2).

The establishment of a three-dimensional anlageplan of the female disc showed that this region does not form adult structures in normal development (Table 1, series 4). Therefore, this region is believed to be the RMP in the mature female disc (Figure 9).

In situ, male tra/tra-genital structures were obtained only from irradiations up to the middle of the second instar. My results show that the formation of male genital structures can be induced even towards the end of the larval development provided that the clone has enough time to grow prior to metamorphosis. Three alternative hypotheses are discussed to account for the requirement for a minimum number of cell divisions in order to revert the phenotypic sex of a clone.

The "repressed" female genital primordium (RFP) was localized by indirect evidence in the posterior half of the ventral sheath of the male disc. This region does not form any imaginal structures, and its site coincides exactly with that of the female genital primordium of intersexual dsx^D-discs.

The genital disc of XX;dsx^D/+ animals, which have male and female genitalia side by side, consists of three major regions (Figure 6). These regions were isolated by fragmentation. After metamorphosis in host larvae, they revealed themselves as the primordia for the male genitalia, the female genitalia, and the analia (Table 4 and Figure 21).

The developmental fate of particular regions of the female and the dsx^D-genital disc was followed by direct observation during evagination (Figures 28 and 29).

With respect to the "repressed" primordia the following general conclusions can be drawn:

- 1) Male and female genital discs of mature larvae carry a "repressed" primordium of the opposite sex (RFP/RMP) which, normally, does not develop genital structures.
- 2) RMP and RFP occupy defined regions in the disc.
- 3) Development in the RMP is slowed down although its cells are able to undergo mitotic recombination throughout larval development and remain competent to react to a change in their genetic sex.
- 4) Certain genetic constitutions (e.g. XX;dsx^D/+, XX;ix/ix, gynanders) allow both genital primordia to develop and to differentiate adult structures.

I. INTRODUCTION

In Drosophila, sex is determined by the ratio of X-chromosomes (X) to autosomes (A). XX;AA animals are females and X(Y);AA animals are males. The Y-chromosome does not play a role in the formation of phenotypic males. Besides the ratio of X-chromosomes to autosomes, several autosomal genes, such as transformer (tra, Sturtevant, 1945), double sex Dominant (dsx^D, Denell and Jackson, 1972) and double sex (dsx, Hildreth, 1965) play a key role in sex determination. The mutant state of these genes is known to interfere with sexual differentiation. Since the difference between a normal male and a normal female is one X versus two X's, the two sexes have identical genetic information. Nevertheless, males and females exhibit a clear sexual dimorphism which must have resulted from differential gene activity. Therefore, the investigation of sexual dimorphism can provide information about mechanisms of establishment and maintenance of developmental programs in general.

The terminalia of Drosophila, i.e. genital and anal apparatuses, which are produced by the genital imaginal disc were chosen for study because they show the most pronounced differences between the sexes (Figures 1 and 2).

Recent studies of gynandromorphs have revealed that male and female genitalia derive from two different regions at blastoderm (Nöthiger, Dübendorfer, and Epper, 1977; Schüpbach, Wieschaus, and Nöthiger, 1978). At this stage male and female animals carry three terminal primordia; the anal primordium occupies the posterior-most region, the female genitalia are located most anteriorly, and the male genitalia map in between.

However, in the adult fly derivatives of only two of these three terminal primordia have been found, namely one set of male or female analia plus either a male or a female set of genitalia. Thus, one has to conclude that in each sex one of the two genital primordia is prevented from forming recognizable adult structures. In this paper, the non-developing genital primordium will be called the "repressed male genital primordium" (RMP), or the "repressed female genital primordium" (RFP).

The mutant transformer (tra) changes XX;tra/tra animals into phenotypically normal, but sterile males (Sturtevant, 1945; Seidel, 1963). Wieschaus and Nöthiger (in preparation) made a detailed analysis of the role of transformer in the development of the terminalia. Clones homozygous for tra were induced by mitotic recombination in XX;tra/+ females. Male genital structures, derived from tra/tra clones, were obtained up to the middle of the second larval instar, but not later. As a rule, the appearance of a male genital clone was accompanied by a complete set of female genitalia. Wieschaus and Nöthiger concluded that: i) the female larva contains imaginal cells in the terminal anlagen which, when homozygous for tra, can form male genital structures; ii) the male structures must derive from cells of the male genital primordium and not from transformed cells of the female genital primordium.

The clonal analysis in situ led to the following questions:

1. Is it possible to localize the male genital primordium in the female disc?

In order to answer this question I tried to localize the site of the male tra-clones in the female disc. In addition, a three-dimensional anlageplan of the wildtype female disc was established.

2. Why can phenotypically male clones only be obtained up to the second instar? Is this the time when the sexual pathway becomes irreversibly determined? Or is a "perdurance effect" (A. Garcia-Bellido and Merriam, 1971a) involved in the action of the transformer gene?

This would mean that the products of the tra⁺ allele, which were present in the tra/+ clone mother cell, are still available and capable to maintain the female pathway until they are diluted out after a certain number of cell divisions.

To distinguish between the two alternatives, female genital discs heterozygous for transformer (tra/+) were irradiated in the late third instar and cultured in vivo for some time to allow for additional cell divisions before being forced through metamorphosis.

3. Is it possible to find a "repressed" female genital primordium in the male disc?

A particular region of the male disc is known not to give rise to any adult structures and therefore will be called a "silent region" in the anlageplan (Ehrensperger, 1972). In order to find out whether this "silent region" represents the "repressed" female genital primordium I tried to induce female genital clones in male animals.

4. The last goal of the present investigation was to study the organization of the genital disc in intersexual genotypes which produce male and female genital structures side by side in the same animal.

II. MATERIALS AND METHODS

1. Culture conditions and general techniques

All animals were kept at 25°C on standard food containing cornmeal, sugar, agar, yeast, and Nipagin. The age of larvae is given in hours after egg laying (h AEL). Cutting and dissection of imaginal discs were done with tungsten needles. Discs and disc fragments were transplanted according to the technique of Ephrussi and Beadle (1936). The metamorphosed implants were isolated and mounted in Faure's solution. When the inner genitalia were stained with orcein, the preparations were afterwards washed on the slide with absolute alcohol and covered with Euparal. Finally, they were studied under a compound microscope and drawings were made with a camera lucida.

2. Induction of male clones in the female genital disc

Mitotic recombination was induced in the following two genotypes:

I. $y^+ w; Dp(1;3)sc^{J4}, y^+ M(3)i^{55}/tra$

II. $y^+/y; Dp(1;3)sc^{J4}, y^+ M(3)i^{55}/th\ st\ tra\ cp\ ri\ p^P$

For a description of mutations and chromosomes see Lindsley and Grell (1968). $M(3)i^{55}$ was described by Morata and Ripoll (1975). Mitotic recombination proximal to $M(3)i^{55}$ and tra yields $M^+ tra/tra$ -clones which in genotype I were marked with yellow (y).

For irradiation of larvae, eggs were collected for 4 h, the larvae were washed out of the food with saturated sugar solution (Nöthiger, 1970), transferred onto a fine Nylon net, washed with distilled water, dried with filter paper and covered with 1 mm plexiglass. A Siemens Dermopan-2 X-ray machine was used with the following parameters: 50 kV; 25 mA; 0.3 mm Aluminium filter; 213 rads/min; focus distance 30 cm. A total dose of 2000 rads was applied.

3. Test for irreversibility of determination of the female pathway (Series A)

Larvae of genotype I were irradiated with 2000 rads towards the end of the third larval instar (144 + 4 h, Minute time). A scheme of the experimental procedure can be seen in Figure 13. The irradiated genital discs were removed

shortly before puparium formation and transplanted back into 74 ± 1 h old wild-type larvae. After metamorphosis of the hosts, the implants were recovered, mounted, and inspected for the presence of male genital structures. This prolongation of the larval period should allow for additional cell divisions. In case of a perdurance effect of the tra⁺ product, male genital clones could now reappear. Irreversible determination, on the other hand, should not be altered by this procedure.

4. Localization of the male genital clones in the female genital disc

4.1. Early irradiation (Series B)

Larvae of genotype I were irradiated with 2000 rads towards the end of the first larval instar (at 54 h, Minute time). In order to find out the origin of possibly induced male tra-clones, the discs were removed in Ringer's solution shortly before metamorphosis and cut into two defined fragments. In a first set (cutting type a), the discs were cut into an anterior and a posterior half; in a second set (cutting type b), the dorsal and the ventral disc epithelia were separated (Fig. 14). Each fragment was injected into a mature wildtype larva for metamorphosis.

4.2. Late irradiation and subsequent in vivo culture (Series C)

After series A had shown that tra-clones could be induced and manifest their male phenotype up to the end of the third instar, the following procedure was chosen for series C: In order to produce a higher frequency of clones than in series B, larvae of genotypes I and II were irradiated with 2000 rads in the second half of the third instar (120-150 h, Minute time). Shortly before puparium formation, the genital discs were removed and injected into one day old wildtype females where they were cultured for 10 - 14 days. This should provide enough time for the clones to grow so that their presence might become recognizable in the cultured disc. A scheme of the experimental procedure is shown in figure 15.

After the culture period, the discs were removed from the adult hosts and examined under the dissecting microscope. Their shape was recorded, and drawings and photographs were made of those discs that showed abnormal morphology, presumably resulting from a growing male clone. The region containing the additionally grown tissue was cut out of the disc and injected for metamorphosis into a mature wildtype female host larva. The rest of the disc was transplanted into a separate larval host. Cultured discs of normal shape were usually reinjected into host larvae without prior fragmentation.

5. Anlageplan of the female genital disc

The interpretation of my results required a detailed anlageplan of the female genital disc. Littlefield and Bryant (1979a) had constructed a two-dimensional fate map of the female genital disc. Since they did not separate the dorsal disc epithelium from the ventral disc sheath, the fate of the dorsal epithelium remained unclear. Therefore, my analysis concentrated on the fate of the dorsal sheath, with the aim of constructing a three-dimensional anlageplan of the female disc.

Discs of mature wildtype larvae (115 ± 5 h after egg laying) were cut into defined fragments which, in order to reveal their developmental fate, were transplanted into mature host larvae for immediate metamorphosis. Five different fragmentation series were made (see Tab. 1).

Series 1 and 2 dealt with the fate of the posterior part of the dorsal sheath. In series 1, the posterior third of the dorsal sheath (fragment A1) was separated from the rest of the disc (fragment R1). For series 2, first a horizontal cut was made anterior to the midline (see Tab. 1). Then, the posterior part (fragment A2) was separated from the ventral epithelium along the lateral edge, and finally, the two fragments were separated along the posterior edge.

Series 3, 4, and 5 dealt with the fate of the anterior part of the dorsal sheath: In series 3, a rhomboid region (fragment S3) comprising the thickened medial anterior part of the dorsal sheath was separated from the rest of the disc (fragment R3). In series 4, the two disc epithelia were carefully separated along the anterior edge. Subsequently, the dorsal sheath was cut at a horizontal level, slightly more anterior to the cutting-line of series 2 (see Tab. 1). In series 5, the anterior lateral margin was cut off from the left and right side of the disc in order to see whether, as a consequence, any structure would be removed or reduced in size. Usually, a rather large part of the anterior dorsal sheath was removed, too. Only the larger remaining fragment (R5) was transplanted.

6. Reconstruction of the female genital disc

Continuous series of paramedian sections through the genital disc of wild-type female larvae were made according to the method described by Eichenberger-Glinz (1979). Mature larvae were injected with cold fixative (2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4, containing 5-6% sucrose). After injection, the larvae were transferred into a small petri dish containing the same fixative. After 30 minutes in the cold fixative, the larvae were cut into halves and left in the fixative for an additional 30 minutes. After rinsing in the same buffer, the tissues were postfixed

(1% osmium tetroxide in cacodylate-sucrose buffer) for 1 h at 4°C, followed by 2-3 h at room temperature. The discs were then freed from larval tissue and embedded in Epon 812 (Luft, 1961). Sections were cut on a Reichert ultramicrotome. The 2.5 μ m sections were mounted in Epon and studied with a compound microscope under phase contrast.

Camera lucida drawings were made of a complete series of histological sections. The drawings were copied onto cardboard and cut out along the perimeter. All cardboard sections were glued together thus forming a three dimensional reconstruction of the disc.

7. Origin of female clones in the male genital disc

For the production of female clones in a male genital disc, the mutation double sex (dsx, Hildreth, 1965) was used. Male flies (XY;AA) as well as females (XX;AA) homozygous for dsx develop abnormal male and female genital structures side by side. XY; dsx/+ larvae, which are normal males, were irradiated with 2000 rads at 50 $\pm$ 2 h after egg laying or with 3000 rads at 66 $\pm$ 3 h after egg laying. Mitotic recombination proximal to dsx should produce clones homozygous for dsx. If such a clone produced female genital structures in addition to a complete set of male genitalia this would be taken as evidence of a "repressed" female genital primordium in the male disc.

8. Organization of the XX; dsx^D/+ genital disc

8.1. Origin and maintenance of the mutant dsx^D

The mutation dsx^D, originally called Hermaphrodite (Hr) was found by Gowen (1942) and later identified as an allele of dsx by Duncan and Kaufman (1975). The mutation transforms XX; dsx^D/+ animals into sterile intersexes which exhibit abnormal male and female elements in their terminalia. The mutation is kept in balanced stocks,

either $x / T(1;3)OR^{60}, y / dsx^D Sb e / Ubx^{130}, Ubx^{130} e^s$
or $x, y / T(1;3)OR^{60}, y / dsx^D Sb e / TM3, sbd^1 Ser e$

8.2. Morphology of dsx^D-genital discs

In this report the term "dsx^D-genital disc" denotes discs of the genotype XX; dsx^D/+. For studying the morphology of the dsx^D-genital disc, animals of the genotype XX; dsx^D Sb e / Ubx¹³⁰e^s and XX; dsx^D Sb e / TM3, sbd¹ Ser e could be selected from the stocks because their larvae had

small gonads and ebony anterior and posterior spiracles. The genital discs were removed and inspected. Photographs and drawings were made of abnormally shaped discs. As a control to check their genotype, some of these discs were transplanted for metamorphosis into mature larvae.

8.3. Reconstruction of the dsx^D -genital disc

The same procedure was used as described for the wildtype female disc (see chapter II/6, p. 6).

8.4. Anlageplan of the dsx^D -genital disc

Genital discs of $XX; dsx^D/+$ mature larvae (115 $\pm$ 5 h after egg laying) were dissected in buffered Ringer's solution and their morphology was recorded. As Figure 6 shows, the dsx^D -genital disc consists of three major regions. I tried to separate these three regions by cutting the disc into two or three fragments according to Figure 21 and Table 4 (cutting type b). In a first series I attempted to separate regions I and II as cleanly as possible, which meant that region III was sometimes split between the two fragments. These preparations, therefore, were not used for the localization of the analia. For this purpose, region III was isolated either by cutting the disc carefully into the three regions, or by making only two fragments so that region III was either together with region I or II (see Table 4). After fragmentation of the disc, the pieces were injected for metamorphosis into mature wildtype female larvae (115 $\pm$ 5 h after egg laying).

9. Study of the evagination of normal female and dsx^D -genital discs

The evagination of the genital disc of wildtype females and of $XX; dsx^D/+$ animals has been studied by dissecting metamorphosing pupae at different times after puparium formation. The developing terminalia were isolated and then studied under the stereo microscope at 1000 fold magnification. Drawings were made from the ventral, dorsal, anterior, posterior, and lateral views.

The normal genital disc was studied at 0, 2, 6, 12, 16, 20, 24, 36, 42, 52 h, and the dsx^D -disc at 0, 3, 8, 14, 18, 21, 24, 32 and 52 h after onset of puparium formation (hAPF).

III. MORPHOLOGICAL DESCRIPTIONS

1. The terminalia of *Drosophila melanogaster*

According to Freeborn (1924), the terminalia of *Drosophila melanogaster* comprise all the imaginal structures posterior to the seventh abdominal segment. These include the external and internal elements of the genitalia and the analia. All elements are formed by one single genital disc which may have arisen by fusion of the imaginal anlagen of the terminal segments (Dübendorfer, 1971).

1.1. The male terminalia

The morphology of the male terminalia has been described by several authors (Gleichauf, 1936; Hadorn et al., 1949; Ferris, 1950; Miller, 1950; Ursprung, 1959; Hadorn, 1966; Bryant, 1978). A drawing of the male terminalia is presented in Figure 1.

The external, strongly chitinized structures (Figure 1a) comprise the 2 lateral anal plates, the genital arch, the posterior lobe of the genital arch, the lateral plates, the claspers and the complex penis apparatus which is connected to the internal structures. These are the sperm pump, the ejaculatory duct, the paragonia, and the vasa deferentia (Figure 1b). The hindgut, the connection between the anus and the rectal bulb of the gut, is also produced by the genital disc.

The external genitalia are bilaterally symmetric and, except

for the penis apparatus, carry a number of bristles arranged in a characteristic pattern. The unique morphology of each male genital element makes it possible to identify them even when only a small piece of an entire apparatus is present.

1.2. The female terminalia

The female terminalia have also been described extensively (Gleichauf, 1936; Hadorn and Gloor, 1946; Miller, 1950; Ferris, 1950; Ursprung, 1959; Mindek, 1968; Bryant, 1978). A drawing of the female terminalia is presented in figure 2.

The external terminalia (Figure 2) comprise the dorsal and ventral anal plates, the 8th tergites, and the vaginal plates which enclose the vulva. The vagina, uterus, parovaria, spermathecae, seminal receptacle, and the common and lateral oviducts form the internal genitalia (Figure 2). The hindgut is also produced by the genital disc. As in males, the female terminalia are bilaterally symmetric. The external genitalia also show a very characteristic morphology. But unlike the male genitalia, only a relatively small fraction of the external elements is covered with bristles and some of these are small and very lightly pigmented.

2. The genital disc of *Drosophila melanogaster*

The bilaterally symmetric male and female genital discs are located ventrally in the 8th abdominal segment just anterior

to the anus of the larva. A single thick stalk connects the medial part of the posterior edge of the disc with the larval epithelium. The genital discs can already be found in 22-25 h old embryos (Laugé, 1968). The cells of the genital discs divide continuously during the last two larval periods (Madhavan and Schneiderman, 1977; Dübendorfer, 1977), but male and female discs cannot be distinguished before the end of the second instar (Laugé, 1968). They reach their full size shortly before metamorphosis (120 h AEL).

The location of the male and the female disc within the larva is the same, but the male disc stands somewhat more upright whereas the female disc lays flatly on the larval epithelium (Littlefield and Bryant, 1979a). As a convention I will call the edge with the stalk posterior and the opposite edge anterior. Consequently, the sheath which contains the anal primordium becomes dorsal, and the sheath opposite to it ventral (Figures 4d, 5c).

2.1. Morphology and fate map of the male genital disc

Photographs of the male disc are presented in Figure 4. A three-dimensional fate map of the male genital disc was constructed by Ehrensperger (1972) and is shown in Figure 8.

The dorsal sheath of the disc starts at the posterior edge with a thick single layer of columnar epithelial cells and gets thinner anteriorly (Figure 4e and 10). According to Ehrensperger this thickened region is the anlage for the anal plates. The anterior half of the dorsal epithelium consists of flat epithelial cells. More anteriorly the thin dorsal sheath joins a very thick region of the disc that seems to be multi-layered and

contains a large mass of adepithelial cells in the anterior region. According to Ehrensperger (1972) this region gives rise to all internal genital structures and the penis apparatus (Figure 8). In the sagittal section, the disc epithelium gets thinner on the ventral side and leads into the ventral sheath where it thickens again in the posterior half close to the stalk which connects the edge of the ventral sheath to the larval epithelium. This thicker medial region in the posterior half of the ventral sheath did not give rise to any imaginal structures and is therefore called a "silent region" in the anlage-plan of the male genital disc (Figure 10). Viewed from the ventral side, the disc lumen is clearly visible and the thick region in the anterior part turns out to consist of two lateral-anterior bulbi (Figures 4b and 10). On each lateral side this region merges into a lateral lobe which leads down almost to the posterior edge of the disc (Figure 4). This lobe is the anlage for the clasper, the lateral plate, and the genital arch (Figure 8). The anlage of the genital arch is split and located in the posterior end of each lateral lobe. During evagination, the lateral primordia of the genital arch fuse on the dorsal side of the disc and thereby form the horseshoe-shaped genital arch (Ehrensperger, 1972).

2.2. Morphology of the female genital disc

Photographs of the female disc are presented in Figure 5. A parasagittal section through the female disc (Figure 5c and 10c) shows that, as in the male disc, the dorsal sheath starts with a thickened epithelium from the posterior edge and gets thinner towards the midline. But even at the thinnest point the cells are cuboidal. The dorsal sheath thickens again in the anterior third (Figure 5b), bends around the

anterior margin and leads into the ventral sheath which is a thick columnar epithelium and contains a mass of adepithelial cells on its ventral side. The thick stalk connects the genital disc in the middle of the posterior edge to the larval epithelium.

The developmental fates of the different regions of the female disc are presented in Figure 9 and described in paragraph IV 3 (three-dimensional anlageplan of the female genital disc (p. 27)).

2.3. Reconstruction of the female genital disc

For a better understanding of its morphology, the female genital disc was reconstructed after camera lucida drawings of a complete series of paramedian sections, kindly supplied by Lynne Littlefield. Photographs of the model are presented in Figure 11.

IV. RESULTS

1. Induction of male clones in the female genital disc

1.1. Test for irreversibility of determination of the female pathway (Series A; p. 4 and Figure 13, p. 84)

None of 53 genital discs that were irradiated at 144 ± 4 h (Minute-time) exhibited any obvious changes in their morphology when they were removed from the irradiated larvae at 160 ± 10 h (Minute-time). After transplantation into 74 h wildtype host larvae, 35 discs produced metamorphosed structures that could be analyzed. In a first group (15 of the 35 cases), intact discs had been transplanted into the 74 h host larvae. In a second batch (20/35 cases), the discs had been cut into two sagittal halves before transplantation.

Six of the 35 analyzed genital discs produced a clone of male genitalia or analia genetically marked with yellow. In two cases a fraction of the penis was found, twice a small distal part of a dorsal paramere was produced, and the two remaining clones comprised 2 and 4 clasper teeth, respectively. All six clones were very small. This shows that cells of the repressed genital primordium are dividing in the 3rd larval instar and that their phenotypic sex can still be changed at that stage provided they have enough time for development before metamorphosis (see Figure 13).

In the analìa, in 18 of these 35 cases, genetically marked yellow (y) bristles were found. The results are presented in Table 3. In four cases the yellow (y) clone comprised exclusively male

bristles (discs 1-4). Six times a yellow clone marked only female anal bristles (discs 5-10). In the other eight cases, both male and female bristles were marked. These eight cases are assumed to represent independent inductions of a male and a female clone (footnote¹). This means that a total of 26 anal clones was observed among the 35 discs. Twelve of the 26 clones were yellow male tra-clones. The other 14 clones derived from distal mitotic recombination and exhibited a yellow female phenotype.

In mosaic anatalia, the formation of male anal tissue is usually associated with a corresponding reduction in the female anal plates (Nöthiger, Dübendorfer and Epper, 1977; Wieschaus and Nöthiger, in preparation). Thus, male and female anal plates are assumed to replace each other. The results of series A partially diverge from this. Only in four of the eight discs which produced a male tra-clone in the anal plates, the formation of a small male clone comprising 2-4 bristles was associated with the absence of a small amount of female tissue (Table 3, discs No. 1, 2, 13, 16). In the other four discs (No. 11, 12, 14, 15) an excess of male and/or female bristles was observed. This may be due to regenerative growth resulting from large amounts of cell death after the heavy irradiation with 2000 rads (Haynie and Bryant, 1977). Those cases where the discs were cut into two sagittal halves before culture are not taken into account for this consideration (see Table 3, discs No. 3, 4, 17, 18) because most of the sagittal halves started to duplicate during the in vivo culture.

footnote¹: Mitotic recombination proximal to the locus of transformer yields male tra-clones genetically marked with yellow (see p. 4). Mitotic recombination distal to the locus of transformer produces a yellow (y) clone in the female pattern. Although the difference between the expected value (3.7) for independent induction of a male and a female clone, and the observed number of cases (8) is insignificant only at the 1% level of confidence, the eight cases are assumed to have resulted from two independent events.

It is of special interest to compare the frequency of male tra-clones formed in the anal primordium and in the "repressed" male genital primordium. As mentioned before, six of the 35 discs contained a male genital clone, compared to twelve discs with a male anal clone. Therefore, at 144 ± 4 h Minute-time, male clones arose twice as frequently from the anal primordium as from the "repressed" male genital primordium.

In four cases the anal clones were accompanied by a male genital clone (twice clasper, and twice penis material see Table 3, discs No. 3, 10, 15, 16). According to Wieschaus and Nöthiger (in preparation) the latter two cases could represent overlapping clones between penis and anal plates. But because of the small number, all four cases might represent two independent inductions (expected value: 2.05).

1.2. Localization of the male genital clones in the female disc

1.2.1. Early irradiation (Series B ; p. 5 and Figure 14, p. 85)

In series B, irradiated genital discs were cut into two fragments which were injected each into host larvae for metamorphosis. By varying the cutting-line I tried to map the region containing the cells of the "repressed" male genital primordium.

In this series, 62 of 89 genital discs which were cut into two fragments before metamorphosis could be analyzed. They yielded three clones. Their male genital derivatives are listed in Table 2 (Series B). In 54 of the 62 analyzed cases, the disc was cut either into an anterior and a posterior half or an anterior fragment comprising two thirds and a posterior piece with one third of the disc (see Figure 14, cutting type a). Two of the 54 discs produced a male genital clone marked with yellow

(y). In both cases, the male structures derived from the fragment containing the anterior half of the disc. One clone was very small and produced only a distal part of a dorsal paramere; it was accompanied by yellow male bristles on the anal plates. No obvious change in the morphology of this disc was noticed when it was cut into an anterior and posterior fragment. In the second case, the anterior half of the dorsal sheath of the mature genital disc was abnormally shaped, showing additional epithelial tissue in this region. Here, the anterior fragment produced - in addition to the expected female genital structures - a large clone comprising elements of the lateral plate, posterior lobe, clasper, apodeme, and hypandrium with hypandrial hairs, but no penis elements.

A small series of eight cases was studied where the disc was cut into a fragment containing the thick ventral epithelium and a fragment with the thinner dorsal sheath (see Figure 14, cutting type b). One yellow male genital clone was found; it originated from a fragment containing the dorsal sheath of the disc. The clone comprised a reduced clasper and a small part of the hypandrium covered with hypandrial hairs.

The identical site of origin of these three clones already suggests that the anterior half of the dorsal sheath contains the cells of the "repressed" male genital primordium. Since large clones were needed at high frequency in order to recognize male genital tissue in the female disc, the new procedure of series C was chosen.

1.2.2. Late irradiation and subsequent in vivo culture (Series C; p. 5 and Figure 15, p. 86)

A total of 261 genital discs were dissected from larvae irradiated at 120 - 150 hM and subsequently cultured for 10-14 days in adult wildtype females. Of these discs, 224 survived

the in vivo culture, and 142 successfully underwent metamorphosis in a larval host.

Of the 224 discs which survived the in vivo culture, 33 were abnormal in morphology. In 14 of these 33 discs, the shape was distorted. These discs were probably degenerating, and the few which metamorphosed yielded reduced terminalia. But the remaining 19 discs had additional tissue, which was clearly recognizable, presumably representing a male genital clone (see Figure 15, 16a, 19a). The outgrowth was always found in the anterior half of the dorsal sheath. This additional tissue was cut out of the disc and transplanted into a mature wildtype larva. The rest of the disc, which was transplanted into a different larva, gave rise to a more or less complete set of female terminalia. Only 11 of the 19 implants exhibiting an outgrowth metamorphosed, but all 11 produced male genital structures. The genital derivatives formed by these clones are listed in Table 2 (Series C). The male genital structures were genetically marked with yellow (y) when genotype I was used (Table 2, clones No. 4, 9, 11, 12).

Some of the clones were quite large and included genital arch, clasper, lateral plates, and parts of the penis apparatus. Except for the ventral parameres, all other elements of the male genital apparatus were found. Some clones were very small and e.g. comprised only one hypandrial bristle and a reduced penis (clone No. 10). Clasper, hypandrium, and lateral plates were found most frequently. Penis, penis mantle, and dorsal parameres appeared in 4 cases. The clones sometimes also comprised internal genital structures: sperm pump was formed in 5 cases, and 3 times ejaculatory duct was found. In 3 cases, the appearance of a male genital clone was associated with the formation of a clone in the anal plates (see Table 2, Nos. 4, 9, 14).

The outgrowths of clones No. 8, 13 and 14 were small and the discs were therefore not fragmented prior to transplantation.

The female genital set was never complete. Usually, both vaginal plates had a reduced number of teeth, the 4-6 small bristles on the 8th tergites were occasionally absent, and sometimes none or only one spermatheca was produced. These types of reductions were also found in the derivatives of uncut genital discs which did not form male clones.

As mentioned before, all male genital clones were found in the anterior half of the dorsal sheath of the female disc. The size of the outgrowth corresponded to the amount of male genital structures found after metamorphosis. Moreover, the particular positions where the clones were growing in the discs (e.g. lateral or medial part of the dorsal sheath) seemed to correlate with the types of male genital structures produced. This is shown in the following description of the morphology of the outgrowths and the structures found in some metamorphosed clones.

Description of clone No. 12 (Figure 16)

The lateral view of the cultured genital disc (Figure 16a) illustrates the position of the three primordia within the genital disc: Rudiments of the stalk can be seen in the posterior region of the ventral disc epithelium. The female genital primordium comprises the entire ventral sheath ($\varnothing$ Gt). The anterior half of the dorsal sheath has developed into a large male genital clone with a lobe pointing in the posterior direction. The anlage of the anal plates (Ap) is recognized as a thickening in the posterior half of the dorsal sheath. In principle, the same arrangement of the three primordia was observed in the dsx^D -disc (Figure 6c). In the dorsal view of Figure 16a, one can recognize the left and right anlagen of the anal plates in the posterior half of the disc (lAp, rAp). The ventral disc epithelium - the anlage for the female genitalia - is lying underneath the focal plane ($\varnothing$ Gt). The male tra genital clone is formed in the anterior half of the dorsal sheath (σ tra-clone). Most of the additionally grown tissue is located in the right lateral half of the disc, forming a short lobe facing posteriorly. In addition, the clone also extends over the sagittal midline towards the left margin.

After metamorphosis, the male clone comprised one clasper, a lateral protuberance, a reduced hypandrium, an apodeme a sperm pump, and an ejaculatory duct (Figure 16b, c) and Table 2). In a normal male genital disc (Figure 8), the clasper and lateral protuberance derive from the lateral anterior region; hypandrium, apodeme, sperm pump, and ejaculatory duct arise from the medial anterior part. This corresponds to the position of clone No. 12 which occupied the right lateral and the middle region of the anterior part

of the dorsal epithelium. The pattern of the male elements is quite normal. The different types of bristles and structures are clearly identifiable. For example, as in normal development, 8 blunt and about 20 sharp teeth can be found on the clasper of the clone.

The remainder of the disc produced female terminalia. The anal plates (Ap) are slightly abnormal; the spermathecae and most of the thorn bristles (thb) of the vaginal plates are missing (Figure 16 b, c).

Description of clone No. 11:

Figure 18a shows the cultured genital disc from a dorsal view. The outlines of the left and right anal primordia (Apl, Apr) are seen in the posterior half of the disc. Opposite to them, a large male clone is protruding from the anterior margin of the otherwise normal female disc. The major part of the prominent outgrowth is placed approximately in the anterior middle of the disc. In addition, a separate posterior lobe extends to the left lateral side.

The metamorphosed clone comprised one clasper, a reduced genital arch, the hypandrium, one lateral protuberance with the four small hypandrial bristles, an apodeme, one dorsal paramere, and penis material (Figure 18 b, c). In addition, four large hypandrial bristles were produced (Figure 18c). They stand in two pairs whereby each pair consists of a normal plus a somewhat smaller bristle.

Position and shape of the clone resemble the left lateral half of a normal male genital primordium (Figure 8 and 18a), and

in accordance with this, the adult derivatives formed by this clone comprised approximately one half of an external male genital apparatus (Figure 18b, c).

Description of clone No. 5:

Like clone No. 12, this case nicely illustrates the position of the terminal primordia within the genital disc. This is best shown in the lateral view of Figure 19a: The dorsal sheath thickens in the region of the anal primordium, becomes thinner towards the middle of the disc, and extends more anteriorly into a large male genital clone. There is a clear separation between the developing male genital primordium and that of the female which comprises the entire ventral sheath. In the anterior view of Figure 19a, one can see how the male and the female genital primordia lie in two different disc sheaths: the male genital primordium lies in the dorsal, the female genital primordium in the ventral sheath. Figure 19a shows that the outgrowth extends mainly to the left lateral side.

The metamorphosed clone formed a genital arch, lateral plate, and clasper which in a normal male disc derive from the lateral lobe of the male genital primordium (Figure 8 and 19c). Only a reduced hypandrium with the hypandrial phragma were found among the structures which derive from the central part of the wild type male primordium (Figures 8 and 19c). This shows that the male clone, which was located in the left lateral side of the cultured disc, produced mainly structures which also derive from the lateral part of the male genital primordium in a normal male disc.

During fragmentation, the primordium of the analia was separated

from the ventral sheath together with the outgrowth. The remaining part of the disc did not metamorphose.

Description of clone No. 9:

This disc showed the largest outgrowth of all 19 interesting cases. However, the morphology of the additional tissue does not resemble a specific part of the normal male genital disc. Two photographs show the disc in an anterior and a posterior view (Figure 17a). Since the focal plane was chosen in the region of the clone, the anlagen of the anal plates are difficult to recognize in the photograph of the posterior view (compare with drawing in Figure 17a). As in all other cases, the male genital tissue developed in the anterior half of the dorsal disc epithelium. As it could be expected from the large size of the outgrowth, the additional tissue metamorphosed into a large male clone comprising a genital arch, a normal lateral plate, a posterior lobe, and reduced amounts of clasper, penis, penis mantle, hypandrium, apodeme, one dorsal paramere, one hypandrial bristle, one lateral protuberance, a sperm pump, and an ejaculatory duct (Figure 17b, c and Table 2).

The female terminalia which derived from the remaining disc fragment were also normal. Only the spermathecae and some thorn bristles of the vaginal plates were missing. As one can see in the drawing (Figure 17b, c), the ventral anal plate carries a male anal clone of five male bristles.

Description of clone No. 15:

This clone is not listed in Table 2 because it was not recognized in the disc after in vivo culture. The outgrowth must have been very small. This is supported by the small amount of male tissue found in the metamorphosed clone which comprised only three clasper teeth and some abnormal structures of the penis or penis mantle.

1.3. Summary of Series A, B and C

In summary, the results of series A, B and C show:

- i) At least up to the middle of the third larval instar the induction of tra-clones in cells of the "repressed" male genital primordium leads to a change from the female into the male developmental pathway, provided that there is enough time for the development of the male clone.
- ii) The wildtype function of the transformer gene (tra⁺) is required to maintain the female developmental program.
- iii) All male genital clones originated in the anterior third of the dorsal sheath of the female genital disc. Since this region does not form any structures in a normal female (seeanlageplan p. 28), it probably contains the repressed male genital primordium in the female disc.
- iv) As a rule, the derivatives of the clones form a normal pattern.
- v) Size and position of the outgrowing disc tissue in the

"repressed" male genital primordium correlate with the amount and types of the male genital structures found after metamorphosis of this tissue (see description of clones p. 20).

2. Induction of female clones in the male genital disc

In order to obtain female clones in the male genital disc XY;dsx/+ animals were irradiated at 50 ± 2 h or 66 ± 3 h. If a dsx-clone were induced in the "repressed" female genital primordium (RFP) it should give rise to female genital structures. No female genital clones have been found among 700 tested animals. Possible reasons for the failure of this experiment are discussed on page 47.

3. A three-dimensional anlageplan of the female genital disc

In the two-dimensional anlageplan of the female genital disc recently presented by Littlefield and Bryant (1979a) the fate of the dorsal disc epithelium remained unclear. Therefore, five series of fragmentations were made in order to reveal the fate of the dorsal disc epithelium. The results are summarized in Table 1. The data allow us to define the positions of the anlagen of the anal plates, the parovaria, and the spermathecae.

A three-dimensional anlageplan is presented in Figure 9. A general survey of the developmental fates of the dorsal and the ventral disc epithelia is best illustrated in Figure 9c. Except for the parovaria, all genital structures are formed by the thick ventral sheath. The dorsal sheath gives rise to the anal plates and the parovaria.

3.1. Derivatives of the dorsal disc epithelium (Figure 9a)

3.1.1. Localization of the anlage of the anal plates

The results of fragmentation (Series 1 and 2, Table 1) show that the anal plates derive from the posterior part of the dorsal epithelium. In both series the A1 and A2 fragments produced complete dorsal anal plates whereas the ventral plate was reduced, especially in the A1 fragment. In these cases, the rest of the plate arose from the complementary R1 fragment. Therefore, the anlage of the ventral anal plate extends more anteriorly than that of the dorsal plate.

According to Littlefield and Bryant (1979a), the primordium of the anal plates is split into a left and right anlage, and they found that an isolated lateral quarter of the disc produces dorsal plates more frequently than ventral ones (98% versus 88%).

Based on all these data, the primordia of the analia are placed into the two lateral posterior corners of the dorsal disc epithelium. The particular orientation of the anlagen for the dorsal and ventral plates is shown in Figure 9a.

The position of the dorsal and ventral anal primordia is inverted with respect to the orientation of the external genitalia (compare Figure 9a with 9b, dorsal and ventral vulva). During evagination, the inverted orientation of the anal anlagen becomes reversed (p. 31 and Figure 28). The hindgut derives from the medial posterior area, just between the two lateral anlagen of the anal plates.

3.1.2. Localization of the parovaria

The small and fragile parovaria are easily lost during preparation and remain difficult to analyze even after staining with orcein. In series 3 (Table 1), only 2 of the 14 fragments produced parovaria (14.3%). In series 4, the cut through the dorsal sheath was done more anteriorly. As a consequence the parovaria appeared more frequently (41%) and they always derived from fragment R4 (Table 1). In the two-dimensional fate map of Littlefield and Bryant (1979a) the parovaria are localized posteriorly to the spermathecae. Therefore, the exact position of the anlage of the parovaria must be in the anterior third of the dorsal sheath (Figure 9a, c).

3.1.3. Localization of the "silent region"

Except for an occasional spermatheca, the thickened region of the dorsal epithelium anterior to the anlage of the parovaria (Table 1; fragment S4) did not produce any structures. The complementary R4 fragment formed a complete set of female terminalia, except that the parovaria were frequently missing. Therefore, fragment S4 is a "silent region" in the anlageplan

of the female genital disc (Figure 9). It coincides with the area from which the male genital clones originated and thus is assumed to represent the "repressed" male genital primordium (RMP) in the female genital disc (Figures 9 and 16a).

3.2. Derivatives of the ventral epithelium (Figure 9b)

Figure 9b is a modification of the two-dimensional anlage-plan of Littlefield and Bryant (1979a). According to them, the external genitalia derive from the posterior half of the ventral sheath while the anterior half produces the internal genitalia. The soft parts (internal genitalia, vagina, and vulva) occupy the central third along the sagittal midline, and the chitinized external elements (vaginal plates and tergite-8) arise from the two lateral thirds. Littlefield and Bryant (1979a) mapped the anlage of the spermathecae to the anterior edge of the disc. The results of my fragmentation series (Table 1) confirm the anterior position and more precisely assign the primordia of the spermathecae to the ventral epithelium. The exceptional cases where spermathecae were produced by fragments of the dorsal epithelium are probably due to cutting errors along the anterior edge of the disc (Table 1, Series 3 and 4). Thus, the anlage of the spermathecae lies in the central part of the anterior margin of the ventral disc epithelium. The internal genitalia (oviduct, seminal receptacle, and vagina) are arranged along the sagittal midline in the same order as in the adult terminalia.

In order to define the lateral extension of the anlagen of uterus and oviduct, the anterior lateral margin was cut off in series 5 (Table 1). The reduced central part always formed normal uterus and bifurcated oviducts. Since this cut probably eliminated only the edge of the anlage of the uterus and oviduct,

the remaining central part might have regenerated the removed lateral parts within the short period between injection and metamorphosis.

The anterior-most structure in the disc is the "silent" region in the dorsal sheath which overlies the ventral epithelium (Figures 9b, c).

4. Evagination of the female genital disc

A series of drawings of the evaginating female disc is presented in Figure 28. The description starts at the onset of puparium formation and the developmental age of the investigated stages is given in hours after puparium formation (h APF).

By the onset of puparium formation (0 h APF) the evagination of the female genital disc starts with an elongation in the antero-posterior axis (Figure 28: 0 h, 2h, 6 h ventral views). Around 6 h APF the disc, which is by now twice as long, opens along the posterior edge (Figure 28: 6 h posterior, ventral, and lateral views). The dorsal sheath folds out gradually. During this process, the anlage of the dorsal anal plate (An), which lays in the posterior most part of the dorsal sheath, turns inside out (Figure 28: 6 h, 12 h lateral views). Subsequently, the anlage of the analia closes in a short tube which later forms the anal opening with the two anal plates (Figure 28: lateral views at 6 h, 12 h, 16 h, and dorsal and ventral views at 16 h). Around 20 h APF 4 small hemispheres are observed at the free posterior end of the anal tube (Figure 28: 20 h, posterior, dorsal, and lateral views). This has changed already at 24 h APF when the outlines of a dorsal and a ventral half of the tube - presumably representing the developing dorsal and ventral anal plates - can be clearly recognized. It is therefore possible that the four hemispheres observed around 20 h APF are the four quadrants of the anal primordium that were already postulated in previous studies (Dobzhansky, 1931; Wüthiger, Dübendorfer, Epper, 1977; Dübendorfer, K., 1977).

The ventral sheath everts around the posterior edge (see arrows in Figure 28: lateral views at 6 h and 12 h). There, the anlage of the tergite 8 (T) shifts back upwards in the anterior direction, subsequently moves around the lateral edge and encloses the anal tube on the dorsal side of the disc (Figure 28: follow morphogenetic movement of T, in lateral, dorsal, and ventral views of 6 h, 12 h, 16 h, and 20 h). The two protuberances which are left in the posterior middle of the disc form the vaginal plates (Vp) (Figure 28: 20 h, 42 h, 52 h, lateral views).

In contrast, the morphogenesis of the anterior half of the disc which forms the internal structures is not an actual eversion but rather a protrusion of appendages: Small bulges which later turn into the drop-like precursors of the spermathecae (Spt) and parovaria (Pv) can already be seen around 6 h APF in the anterior part and around the middle region of the dorsal sheath, respectively (Figure 28: 6 h, 12 h, 20 h, 42 h, lateral and posterior views). The anlagen of the spermathecae must have moved into their final position within the first 6 h of evagination. Interestingly, they are arranged in a longitudinal tandem and not as two lateral anlagen as one might have expected from their final position in the genitalia. Around 16 h APF the seminal receptacle (R) becomes visible at the anterior tip of the disc (Figure 28: 16 h, 24 h, 42 h, anterior views). By 24 h this anlage elongates into a tube-like protrusion of the uterus. Between 36 and 42 h APF the tube must have elongated tremendously and appears now coiled up in its typical spiral shape. The oviducts (Od) grow out of the lateral vaults at the anterior-lateral margin (Figure 28: 20 h, 24 h, 36 h, 42 h). By 36 h APF the rapidly elongating lateral oviducts exceed all other appendages in the anterior direction. They now establish contact with the developing ovaries and by 42 h the fusion is complete.

From 20 h APF to 40 h APF, all the above mentioned protrusions (Spt, Pv, R, Od) mainly elongate and approach their final shape around 40 h APF (Figure 28: 42 h). This is also the time when the bristles of the anal plates and the vaginal plates are formed. Around 52 h the terminalia appear fully developed, but not yet pigmented (Figure 28: 52 h).

5. Organization of the dsx^D -genital disc

5.1. Morphology of the terminalia of dsx^D -flies

$XY;dsx^D/+$ flies are normal, fertile males. $XX;dsx^D/+$ flies are sterile and exhibit an intersexual phenotype with male and female genital structures side by side in the same animal (Gowen, 1942; Roost, 1978). Figure 3b shows the typical phenotype of the external structures of the terminalia of an $XX;dsx^D/+$ fly: Male and female genital structures are separated whereby in situ the female genitalia are placed ventral to the male genitalia.

The pattern in the male set is always somewhat abnormal: the chitinous parts are slightly shrunken in appearance and the number of bristles is often reduced to about 80% of normal. Nevertheless, the structures can be clearly identified as male genital elements. The severest abnormality was found in the penis apparatus (Figures 3b, 23 and 24). In most cases the apodeme, the ventral paramere, the two hypandrial bristles, and the dorsal part of the hypandrium with its characteristic hypandrial hairs are missing. The lateral protuberances of the hypandrium with the four small hypandrial bristles are present. A reduced ventral part of the hypandrium is produced in about 50% of all flies and is not attached to the penis, penis mantle, and the dorsal paramere. These latter three structures are formed in almost all cases whereby penis and dorsal paramere were again reduced to varying degrees; penis mantle seems to be normal in all cases.

The vaginal plates are always reduced, consisting of a dorsal rest that contains two to six thorn bristles often accompanied by two to three sensilla trichodea. In most cases, the row of

thorn bristles ends with an enlarged long bristle. The 8th tergite is sometimes split by a narrow slit in the dorso-ventral axis. The arch of the 8th tergite encloses the male genital arch to which it is always fused at the dorsal margin.

The inner genitalia (Figure 3a) are also separated into the two sexual sets; they are attached to the corresponding external derivatives. In most cases the two sets of inner genitalia were quite complete. The observed reductions were not restricted to any particular internal element. A few extreme cases have been found with only a small amount of internal structures. Connections between male and female parts were observed, and in some cases a paragonium was attached to the uterus or an oviduct. The paragonia were the only internal genital structures that were sometimes produced in increased numbers. In some cases, a third paragonium was even attached to a female ductus.

Interestingly, the vagina usually enclosed a mass of chitinized yellow pigmented tissue. Upon closer examination these structures were identified as rudimentary elements of a penis apparatus, consisting of a penis, surrounded by dorsal parameres and penis mantle. Since dsx^D -flies also have a reduced penis apparatus including penis, dorsal parameres, and penis mantle in normal position in the male genitalia, these three elements found in the vagina represent a duplicate of a particular part of the penis apparatus. The same phenomenon is observed in the terminalia of XX;dsx/dsx, XY;dsx/dsx and XX:ix/ix flies. The situation was already illustrated in the study of double-sex (dsx) flies by Hildreth (1965), but the duplicated structures were not identified.

A few cases were found where the terminalia of XX;dsx^D/+ animals were reduced to such an extent that only traces of the male

and/or the female genitalia remained.

Like normal males, XX;dsx^D/+ flies always had symmetrically arranged left and right anal plates. However, their drop-like shape resembled neither the wildtype male nor the normal female in appearance. Each of the two plates usually contained about 20 to 30 bristles (Figure 3b). Most anal plates carried one long bristle of the same length as the long bristles in wildtype female anal plates. Of the remaining bristles, some were typically male, some typically female, and some were intermediate in length. In a few exceptional cases, the two anal plates showed the typical male orientation, shape, and bristle pattern. No female long bristle was found. Nevertheless, each of these anal plates bore only 25 bristles.

It appears as if the anal plates of dsx^D-flies carry a mosaic of normal male, female, and intermediate bristles.

5.2. Morphology of the dsx^D-genital disc

The shape of the genital disc of XX;dsx^D/+ animals resembles that of the normal male disc. Parasagittal sections of the dsx^D-disc (Figure 6c), however, reveal two large regions (I and II), and one smaller region (III), whereas the normal male disc has only one large plus two small regions (Figure 10a, ♂ Gt, Ap, SR). Region I of the dsx^D-disc lies in the ventral sheath, region II in the anterior part, and region III in the posterior half of the dorsal sheath.

The dorso-laterally situated region II resembles that part of a normal male genital disc which forms the male genital structures. As in a normal male disc one can recognize two anterior bulbi and two lateral lobes (Figures 4, 6, and 10).

On the one hand, the position of region I corresponds exactly to that of the "silent region" in the male disc (Figures 4d, 6b, and 10), but it is much bigger than this "silent region". On the other hand, the shape of region I resembles the ventral part of a normal female genital disc. This area is the primordium of the female genitalia in the female disc. Its size is slightly larger than that of region I (Figures 5c, 6, and 10).

The position of region III in the posterior half of the dorsal sheath already suggests that it could be the anlage for the analia.

The existence of these three regions becomes even clearer when a complete series of parasagittal sections is considered (Figure 7): in the sagittal section (Figure 7d) only regions I and II are recognizable because region III consists of a left and a right part located in the lateral third of the dorsal disc epithelium. This is best shown in a more lateral section where all the three regions can be seen (Figure 7c). When cut even more laterally, the shape of region II changes drastically because the section now also cuts through the lateral lobe (lL) of region II (Figures 7a, b and 6a).

In summary, the morphology of the dsx^{D} -genital disc already suggests that region II and I might be separate anlagen for the male and female genital structures.

5.3. Fragmentation analysis of the dsx^{D} -disc

5.3.1. Localization of the male and female genital primordia

The developmental fates of the three major regions were determined by cutting the disc into two or three fragments according to Figure 21 and Table 4, and subjecting them to immediate

metamorphosis. In a first experimental series, regions I and II were separated. A maximum of 49 fragments containing region I and 60 fragments containing region II were analyzed. The results, which are presented in Figure 21, show that the male genital structures arose from region II (hatched in Figure 21) whereas the female genital structures were formed by region I (dotted in Figure 21). Photographs of the derivatives of two complementary fragments from the same $\underline{dsx}^D$ -disc are presented in Figure 22.

The separation is very clear except for the formation of two genital elements, the penis apparatus and the paragonia. In the fragmentation analysis, penis was found in 55 of the 60 fragments containing the "male" region II, but also 11 of the 49 fragments containing the "female" region I produced penis structures. 56 "male" fragments yielded penis mantle as did also 14 of the "female" fragments. The rest of the penis apparatus was produced exclusively by "male" fragments. As in situ, some parts were strongly reduced compared to a wildtype apparatus.

Variation of the cutting line cannot be the only reason for this overlap because in most cases, where the "female" region I produced the above mentioned structures of the penis apparatus, these elements were not missing in the corresponding "male" fragment. This observation supports my earlier conclusion that the elements of the penis apparatus found in the female genitalia are a partial duplicate of the penis apparatus of the male genitalia. Figure 23 shows the structures of a normal penis apparatus, and Figure 24, the rudimentary penis apparatus and the duplicated elements in the vagina of a $\underline{XX;dsx}^D/+$ animal. As in situ, in the metamorphosed implants of fragment I, the duplicated part was always located in the vagina; the size of the duplicated structures was smaller and the proximo-distal orientation was

identical to that of the male genitalia.

The other region of overlap concerns the formation of the paragonia (Figure 21): 29 of the 66 fragments containing the "male" region II produced paragonia; but this structure was also formed by 9 of the 49 "female" fragments I. From these 9 cases, 7 of the "male" complementary fragments did not form any paragonia. Only in two cases was one supernumerary paragonium produced. This, then, suggests a variation in cutting and, hence, imperfect separation of the male and female regions in the two fragments. However, the occasional formation of three paragonia in the same fly was also observed in situ of XX;dsx^D/+ control flies.

5.3.2. Localization of the primordium of the analia

A second series with cuts of the types a), b) and c) (Table 4) were made with special care taken for the clean separation of region III, which is located in the thickened part of the posterior half of the dorsal sheath. The results are presented in Table 4.

In 17 of the 20 analyzed cases where the dsx^D-disc was cut into three fragments, each of them comprising only one of the three regions (Table 4, cutting type c), anal plates derived exclusively from region III (fragment G). In three cases fragment E and/or fragment F also produced a small amount of anal plate tissue.

In some cases the anal plates were reduced or the pattern was disturbed, and occasionally trichomes were found among otherwise typical dsx^D anal bristles. When anal tissue was produced from fragments that should not have contained region III, the amount was always small. This imperfect separation might have been caused by variations in cutting.

Altogether, in 46 of the 51 tested discs listed in Table 4, anal plate structures were exclusively produced by those fragments that contained region III (fragments B, D, and G). Therefore, region III is regarded as the anlage of the analia in the $\underline{dsx}^D$ -genital disc.

5.4. Evagination of the $\underline{dsx}^D$ -genital disc

5.4.1. General description of the evagination

A series of drawings of the evaginating $\underline{dsx}^D$ -disc is presented in Figure 29. The evagination of the male and female genitalia in the $\underline{dsx}^D$ -disc very much resembled that seen in wildtype male and female genital discs.

The evagination begins with an elongation of the $\underline{dsx}^D$ -disc along the antero-posterior axis. Three hours after the onset of puparium formation (3 h APF), the disc starts to open along the posterior edge between the primordia of the analia and the female genitalia (Figure 29: 3 h, dorsal and lateral views). At the same time the onset of the evagination of the internal male genitalia can be observed in the anterior part of the disc. The anlagen of the paragonia (Pg) start to protrude in the anterior direction, and a demarcation line appears between them and the rest of the anterior bulbi of the male genital primordium (Figure 29: lateral, anterior, and ventral views of 0 h and 3 h). The evagination of the internal structures of the female primordium is blocked by the presence of the male anterior bulbi (Figure 29: 3 h, dorsal, ventral and lateral views). As a consequence, the evaginating anterior female part turns aside and protrudes ventrally to the male primordium (Figure 29: 8 h, lateral view). From now on, male and female internal structures evaginate parallel to each other in the anterior direction (Figure 29: lateral views of 18 h, 24 h, and 32 h).

The external genitalia of the male and female primordia evaginate in the same way as in wildtype discs. The process is briefly described in the following paragraphs. By 8 h APF the male and the female developing genitalia still share a common lumen (Figure 29: 8 h, dark area in lateral and anterior views), which separates gradually during evagination (Figure 29: lateral view of 8 h, 14 h, and 21 h). At 14 h APF, they share only a short septum in the posterior most part. Interestingly, they never fully separate in that region.

5.4.2. Evagination of the analia

The evagination of the anal primordium also parallels that seen in the normal male and female discs (Ehrensperger, 1972; and chapter III, 4). The anal primordium (An) everts around the posterior edge and moves outwards and anteriorly to form the short anal tube (Figure 29: lateral and dorsal views of 8 h, 14 h, and 18 h). Already at 21 h APF a left-right separation of the anal tube is indicated (Figure 29: 21 h, posterior view). At 24 h APF this separation is clear, and around 30 h APF the two lateral anal plates can be recognized (Figure 29: 32 h, posterior, dorsal, and ventral views). The stages where four quadrants were observed before their fusion into two anal plates (see female evagination p. 31) was not seen in the dsx^D development. But this could have been overlooked. The example drawn at stage 24 h APF shows the two lateral anal plates fused in the dorsal region (Figure 29: 24 h, posterior view). This occurs occasionally and is also seen in the differentiated anal plates.

5.4.3. Formation of the male genitalia

As in normal development, the anlagen of the male genital arch located in the posterior edge of the lateral lobes start to evert at the posterior lateral side, and subsequently move along the outside towards the anal plate tube which becomes enclosed by the genital arch around 24 h APF (Figure 29: lateral and dorsal views of 14 h, 18 h, and 24 h). During this process, the anlagen of the lateral plate and clasper are dragged along. Thus, the orientation of the lateral lobe becomes reverted with respect to the internal genitalia. Finally, by 32 h APF, the lateral plates, the clasper, and the genital arch are arranged in a horseshoe-like lobe around the anal plates and the male genital opening (Figure 29: 32 h, posterior view). By that time the claspers are only indicated as a protrusion of the male genital lobe.

The areas of the anlagen for the penis apparatus, the sperm pump, and the paragonia can be distinguished after 18 h APF (Figure 29: 18 h, dorsal and lateral views). The two paragonia protrude from the two anterior bulbi. Due to the variability in the number of paragonia, their development was difficult to follow. But by 24 h, the areas of the penis apparatus, the sperm pump, the ductus, and the paragonia are clearly delineated. By 32 h, the penis apparatus is more integrated into the external genitalia. The ductus undergoes an enormous elongation during this period (Figure 29: lateral view of 24 h and 32 h) whereas the paragonia enlarge mainly between 32 h and 50 h.

5.4.4. Formation of the female genitalia

As in a normal female disc, the evagination of the external female genitalia starts with the eversion of tergite-8 (T). The question now arises how the tergite-8 will achieve its final location which is peripheral to the male genital arch.

In the XX; dsx^D/+ disc the evaginating lateral edges of the 8th tergites are expected to move around the female and the entire male genitalia to enclose the anal tube. This process was difficult to follow. The lateral parts of the two sets start to evert at the same time (Figure 29: 14 h, lateral view). The tergite-8 presumably moves together with, or alongside, the male genital arch. Only at 24 h APF, when these movements are already completed, can the two structures be distinguished (Figure 29: 24 h, lateral view).

The formation of the internal female genitalia was also difficult to investigate. By 14 h, two prominent bulges, representing the anlagen of the spermathecae, can be detected on the dorsal side (Figure 29: lateral view of 14 and 18 h). The anterior lateral vault of the evaginating female genitalia often showed an additional prominent bulbus that was placed on one or both anterior lateral edges (Figure 29: 14 h and 18 h). Since occasionally, a paragonium was found attached to the female duct, this additional tissue could represent a developing male paragonium.

5.4.5. Additional remarks

The figure drawn at 52 h APF demonstrates that by this time all structures have attained their final morphology. The numerous morphological abnormalities described for the adult terminalia of dsx^D-flies can already be seen at this stage.

The evagination of two spermathecae was observed in the dorsal region of the female genitalia (Figure 29: lateral view of 14h, 18 h, 24 h and 52 h). However, parovaria were never found to evaginate in the dorsal part, or any other region of the female genitalia. The parovaria of a normal female fly are clonally related to the analia (Dübendorfer, K., 1977; Schüpbach et al., 1978) and their anlage is placed between the analia and the "repressed" male genital primordium (Figure 9c). In the dsx^D-disc

the anlage of the parovaria would be completely separated from the female genitalia by the developing male genital primordium. During evagination, the anal plate primordium separates from the female genital primordium and evaginates to the dorsal (male) side. If any parovaria had stayed associated with the anal plates, they may have been overlooked. Alternatively, the parovaria might not be produced at all in the mutant genotype since they also were not found among the dissected control flies (see also Roost, 1978).

5.5. Anlageplan of the dsx^D -disc

A three-dimensional anlageplan of the dsx^D -disc is presented in Figure 10b. The anlageplan is based on the results of the fragmentation analysis (Table 4) and the study of the evagination of the dsx^D -disc (p 40 and Figure 29). A reconstruction of the dsx^D -disc is presented in Figure 12.

In summary, the observations show that:

- i) The dsx^D -genital disc of a mature larva contains all three terminal primordia in a well developed state. After metamorphosis, derivatives of all three primordia can be found in the intersexual terminalia.
- ii) The genital and anal derivatives are always somewhat abnormal; certain elements are missing and a specific part of the penis apparatus appears duplicated in the vagina.
- iii) The positions of the three terminal anlagen of the dsx^D -disc are identical to those in the wildtype male and female genital disc. The primordium of the female genitalia (region I) lies in the ventral sheath. The dorsal sheath contains the male genitalia (region II) in the anterior and the anal primordium (region III) in the posterior part.

- iv) The morphology of the terminal primordia in the disc resembles that of the corresponding wild-type anlagen. Furthermore, the process of evagination reveals that the primordia are organized in the same way as the corresponding wildtype anlagen.

V. DISCUSSION

1. Localization of the "repressed" male genital primordium in the female genital disc

The evidence for the presence of a "repressed" male genital primordium (RMP) in the female genital disc comes from three sources.

First, the induction of tra-clones resulted in visible outgrowths from the female genital disc. When cut out of the disc and sent through metamorphosis, these outgrowths produced male genital structures exclusively. The rest of the disc formed female genitalia and analia. These outgrowths were always observed in the anterior third of the dorsal sheath of the female disc (Figure 16a). Thus this region is assumed to be the "repressed" male genital primordium in the female disc.

Second, for the establishment of an anlageplan of the female genital disc, this same region was cut out of the disc and sent through metamorphosis. Apart from a few exceptions, this region did not form any adult structures (Table 1, Series 4). Therefore, this part is called a "silent region" (SR) in the anlageplan of the female genital disc (Figure 10).

The third line of evidence comes from a comparative analysis of normal and intersexual genital discs. The location of the "silent region" of the female disc coincides exactly with that of the male genital primordium in the dsx^D-disc (Figure 10b, c). In addition, a few cases were found with a severely reduced male genital primordium in an otherwise normal dsx^D-disc.

After metamorphosis in a host larva, these discs produced only a rudimentary male genital apparatus. Figure 25 shows that with increasing reduction of the male genital primordium the morphology of the dsx^D-disc resembles more and more that of the wildtype female disc. Such a sequence of reduction directly points to the site of the "repressed" male genital primordium in the female disc.

Based on these observations, the "silent region" in the anterior half of the dorsal sheath of the female disc is regarded as the "repressed" male genital primordium (RMP).

2. Localization of the "repressed" female genital primordium in the male genital disc

The direct approach to localize the "repressed" female genital primordium (RFP) in the male disc by induction of female genital clones failed. No female clone was found in the terminalia of 700 irradiated XY;dsx/+ males.

Among 420 forelegs of the same flies, only one case was observed where a clearly reduced sex comb was associated with the appearance of female bristles in the 7th transversal row of the basitarsus.

The absence of female genital clones is not understood. On one hand it may not be possible at all to induce dsx-female clones in the male genital disc, on the other hand a dsx-genital clone might require more time or cell divisions for its phenotypic manifestation than were available. This will be tested by using a Minute system with suitable genetic markers and earlier irradiations.

So far, the localization of the "repressed" female primordium is based on indirect evidence. Ehrensperger (1972) found a "silent region" in the ventral epithelium of the male genital disc (Figure 8). The location of the "silent region" corresponds to the site of the female genital primordium of the dsx^D-disc (Figure 10). Thus, the "silent region" of the male disc is assumed to be the "repressed" female genital primordium (RFP).

3. Development of the terminalia in the male, female, and in an intersexual genotype

The presented results show that the mature discs still contain the three terminal primordia which were postulated for the blastodermal anlage of the terminalia (Nöthiger, Dübendorfer, and Epper, 1977; Schüpbach, Wieschaus and Nöthiger, 1978). The principle arrangement of the different primordia is the same for the genital discs of all tested genotypes. But the development of these primordia depends on the genotype of the animal (Figure 26).

In a XX;AA animal, the cells of the terminal primordia develop according to the female genetic program (Figure 26, ♀ pathway). In this case the imaginal cells of the 9th segment fall into the "repressed" state (RMP) and do not produce adult structures.

In a male animal (XY;+/+ or XX;tra/tra) the imaginal cells of the 8th segment form a "repressed" female genital primordium (RFP).

In the intersexual development of XX;dsx^D/+, XX;dsx/dsx,

XY;dsx/dsx, or XX;ix/ix animals, neither of the two genital primordia seem to be repressed. All three terminal primordia develop adult structures, that, however, exhibit various abnormalities.

4. The "repressed" state of the genital primordia

Since mitotic recombination occurred in the "repressed" male genital primordium (RMP) during the second half of the third larval instar (series A and C), at least some cells of the RMP must still be dividing at that stage. However, several observations indicate that the development in the "repressed" primordium is slowed down:

- i) In histological sections (Laugé, 1968), male and female genital discs become distinguishable from each other towards the end of the second instar. Therefore, the male and female genital primordia must have grown differently during development prior to that stage.
- ii) The size of the RMP in a mature female disc is estimated to be smaller than 1/10 of the male genital primordium in a normal male disc. About the same relation holds for the RFP in the male disc. This suggests that the growth is reduced in the "repressed" primordia.
- iii) The smaller size of the RMP is also reflected in the lower frequency at which tra-clones can be induced in the RMP compared to that in the male genital primordium of a XY;tra/+ male (Wieschaus and Nöthiger, in preparation).
- iv) Frequency and size of the tra-clones induced in the RMP remained roughly constant through the second instar,

although variation in size was high at all stages (Wieschaus and Nöthiger, in preparation). In a cell lineage analysis of the genitalia of normal males and pseudo-males (XX;tra/tra), the frequency of clones increased and the clone size decreased with irradiations at later stages (Dübendorfer, K., 1977). This shows that up to the middle of the second instar the mitotic activity in the RMP is low.

- v) At 135 ± 15 hM the frequency of induced tra-clones in the RMP is significantly higher than that observed in situ at 81 hM (0.018). In series C, the frequency was 0.049, which is an underestimate since only metamorphosed cases are considered. If in series C all the 19 cases with an outgrowth are regarded as clone inductions, the frequency is 0.085. In series A, where irradiation occurred at 144 ± 4 h, the frequency of tra-genital clones rose to 0.17. These results suggest an increase in the number of cells in the RMP during the third larval instar, but it has to be taken into account that I used 2000 rads for my experiment whereas Wieschaus and Nöthiger only applied 1000 rads.

Altogether, the arguments i-v show that little mitotic activity occurs until the middle of the second instar but seems to increase in the third instar. This could be explained in two ways.

First, mitotic activity might decrease constantly in the RMP during the first two larval instars so that the number of cells sensitive to mitotic recombination remains constant.

Second, the mitotic behaviour of the cells in the RMP could be similar to that of the abdominal histoblasts (A. Garcia-

Bellido and Merriam, 1971b). This would mean that the cells stay arrested in the G-2 phase of the cell cycle during the first two instars and start to divide only in the third instar. As proposed in the last chapter of the discussion (p. 55), the imaginal cells of the 9th segment (RMP) in a female animal are assumed to be programmed to form a female tergite-9. But with the tendency to reduce the postabdomen during evolution, the capacity to produce this adult structure seems to be lost. Thus, the mitotic behaviour of the RMP might be a relic of a more primitive stage.

5. Do the cells of the female disc become irreversibly determined for the female pathway during the second larval instar?

In situ, male genital clones could no longer be found in discs irradiated later than the mid-second instar (Wieschaus and Nöthiger, in preparation). The results of my experiments, where irradiated genital discs were given additional time for growth prior to metamorphosis (series A and C), show that male genital structures can even derive from discs irradiated in the second half of the third instar. Therefore, the cells of the female genital disc do not become irreversibly determined for the female program at least up to the second half of the third instar (150 hM).

Three possible reasons for the effect observed in situ will be discussed in this chapter.

- i) First, a perdurance effect (A. Garcia-Bellido and Merriam, 1971a) might be involved in the action of the transformer

gene. This means that a minimum number of cell divisions is needed in order to dilute out the tra⁺-product and to reverse the phenotypic sex of the clone. Irradiation after 81 hM does not provide time for enough cell divisions prior to metamorphosis.

- ii) A second hypothesis proposes that a minimal number of unrepressed cells is needed to form a male pattern element in a female disc. We assume that imaginal cells have to be integrated in a "mature positional field" in order to react with imaginal differentiation. Since development in the RMP is retarded, a tra-clone first has to reach a critical size in order to establish an island of mature positional information. Clones which did not have enough time to grow and build up a field cannot respond with the formation of male genital structures.

This hypothesis predicts that in the normally developing female genital primordium of XX;tra/+ animals even tra-clones that were induced at late stages should show up since their cells lie in a "mature field" and can, therefore, react without additional proliferation. The result would be a cluster of "repressed" cells leading to a gap in the female pattern. Even very small clones, comprising only a few cells, could result in a loss of just one or two bristles within the row of vaginal plate bristles.

In fact, Wieschaus and Nöthiger (in preparation) found defects in the vaginal plates presumably caused by tra-clones after irradiations at least up to 102 hM. This is considerably later than the latest time when male genital structures can be induced to form (81 hM).

- iii) A third hypothesis suggests that a retardation of development occurs in the RMP. The cells of the RMP which have a reduced mitotic activity (see paragraph 4: The "repressed" state of the RMP, p 49) might be lagging behind

the development of an unrepressed primordium. According to this hypothesis a growing clone would first have to reach a critical stage in order to become competent to metamorphose. This implies that the use of the Minute technique, where the $\text{tra } M^+/\text{tra } M^+$ cells of the clone are growing faster than $\text{tra } M^+/\text{tra } M$ cells in the rest of the disc, should shift the relative time limit for the latest successful differentiation of male genital structures towards puparium formation. No such shift was observed. According to Wieschaus and Nöthiger (in preparation), the relative time limit is approximately the same when the tra-clones had the M^+/M^+ advantage among the M/M^+ background (81 ± 8 hM; which corresponds to about 68 h in normal development) as when no Minute technique was used (71 ± 2 h). This may rule out the last hypothesis as the only cause for the observed effect.

At present it is not possible to distinguish experimentally between the three hypotheses. They all depend on a minimum number of cell divisions, and they all have their critical effects towards the end of larval development when exact timing gets extremely difficult and the clone sizes become very small. It is even conceivable that the behaviour of tra-clones in the "repressed" male genital primordium is due to a combined effect of perdurance, clone size, and retardation in development.

6. Cell lineage relationships in the genital discs after in vivo culture

According to Wieschaus and Nöthiger (in preparation), the embryonic genital disc contains two clonally separated populations of cells:

penis, penis mantle, and dorsal parameres form a common pool with the analia, whereas the rest of the male genitalia and the female genitalia form the other. Sánchez and Nöthiger (in preparation) were able to induce male genital clones in female flies by changing the ratio of X-chromosomes versus autosomes during the larval development. As in the transformer analysis of Wieschaus and Nöthiger, penis, penis mantle, and dorsal parameres appeared clonally separated from any other element of the male genital apparatus.

However, in series C of my experiments, where the irradiated genital discs were cultured for 10 - 14 days prior to metamorphosis, all 4 clones which produced penis, penis mantle, or dorsal paramere were accompanied by some other elements of the male genitalia (Table 2). One case (clone No. 9) displayed a complete inventory including genitalia and analia, and could, therefore, have derived from two independent clone inductions in the anal and the "repressed" male genital primordium. But two independent events cannot be responsible for all four clones, and we thus have to conclude that in my experiments, the developing clones did not respect the clonal boundary observed in situ.

Since I have used 2000 rads compared to 1000 rads for the in situ-analysis, and since I have cultured the discs for 10 - 14 d in vivo, the results are not necessarily contradictory. The higher dose must have killed a high fraction of the cells (some 85%, according to Haynie and Bryant, 1977), and the culture period may have allowed regeneration to take place. It is known that cells involved in the process of regeneration lose their compartmental commitment (Szabad, Simpson, and Nöthiger, 1979). Such a phenomenon can reconcile my data with those of Wieschaus and Nöthiger.

7. On the evolution of the terminalia

In Drosophila melanogaster, the dimorphism between male and female elements is of different intensity. Relatively subtle differences exist for body size and pigmentation in the abdominal tergites. A more distinct dimorphism is observed in the forelegs of the two sexes. There, the choice is between the formation of two distal-most transversal rows in the basitarsus of the female versus the differentiation of a proximodistally orientated sex comb in the male animal. The primordial cells of the analia have to choose between the formation of two types of anal plates which, in addition, bear different bristle patterns. Finally, the most extreme sexual dimorphism is found in the genitalia. Here, the alternative is not to choose between two patterns, but rather to make a decision whether or not the entire male or female genital primordium will develop recognizable adult structures.

The following section is based on phylogenetical aspects of the terminalia of different Diptera and suggests that there is probably no principle difference between the mechanisms for the establishment of the sexual dimorphism in the genitalia and those in the other mentioned parts of the fly. A hypothetical scheme with the male and female developmental alternatives at four stages of evolution is shown in figure 27.

There is a tendency during evolution of Diptera to reduce the elements of the postabdomen, i.e. the segments posterior to the 5th abdominal segment (Crampton, 1942). Such reductions occurred to a different extent in male and female animals, which, as a consequence, has led to an increase in sexual dimorphism. Two main aspects are illustrated in figure 27. One concerns the reduction of the tergites and sternites in males

and females, the other the tendency of the segmental imaginal anlagen to fuse into one single terminal disc (Dübendorfer, 1971).

The situation visualized for the development of hypothetical primitive Diptera is drawn in figure 27a. These male and female flies are assumed to possess well developed tergites and sternites in the 8th as well as the 9th segment, in addition to their primitive genital apparatus. The genital elements are believed to represent modified sternites (Crampton, 1942). They should therefore derive from the ventral part of the segmental imaginal anlage. Thus, some cells of the primordium of the 8th imaginal segment would be specialized to form a very simple gonoporus in the female. In the male, all cells of this segmental anlage contribute to the formation of an 8th tergite and sternite (T/s-8). The reciprocal situation exists in the 9th segment. In the female, all cells are assumed to form a complete 9th tergite and sternite (T/s-9). In the male genotype some cells form a simple phalloderm (σ^8 Gt) whereas the rest of the primordium produces a 9th tergite and a reduced sternite (T/s-9). Thus, at this primitive stage, the cells had a choice between two adult patterns, as in the analia of Drosophila melanogaster.

The anlagen posterior to the 9th segment give rise to the anal appendages. These segments tend to be reduced already in primitive insects (Crampton, 1942).

A first level of reduction is drawn in figure 27b. Representatives of this level can be found among the Tipulidae. There, male flies have a reduced tergite (t-9) plus a phalloderm (σ^8 Gt) in segment 9, but, as a first step in the reduction process, also the tergite and sternite of the 8th segment are reduced. Analogously, the females have a reduced tergite and

sternite in their 9th segment (t/s-9).

This can be explained by the hypothesis that in a male animal some primordial cells of the 8th imaginal segment do not form adult structures anymore. As a consequence, a reduced tergite and sternite are produced. Since in the females this primordium must give rise to the genital apparatus, only limited reduction can occur in segment-8. In the female terminalia, the tergite and sternite of segment-9 undergo reduction.

The fate of some cells not to contribute anymore to the formation of adult structures in one sex is an extreme form of reduction of the adult pattern. Since these primordial cells might provide positional information necessary for stabilizing the segmental field, they are retained during development of the imaginal segmental anlage (Littlefield and Bryant, 1979b). The following stages show that this reduction tendency increases during evolution and ends with the complete loss of an entire adult segment in one sex (stage 4).

The situation described for Calliphora (Emmert, 1972a, b) and Musca domestica (Dübendorfer, 1971) is representative of the second level of reduction (Figure 27c). In these animals, all terminal anlagen of segments 9-11 are fused into the median genital disc (Dübendorfer, 1971). The anlage of segment-8 consists of the two lateral genital discs in the 8th segment of the male and female larva.

No derivatives of the 9th segmental anlage are found anymore in the female terminalia. In the male, the imaginal cells of segment-9 give rise to the male genital apparatus and the 9th tergite which is called the epandrium.

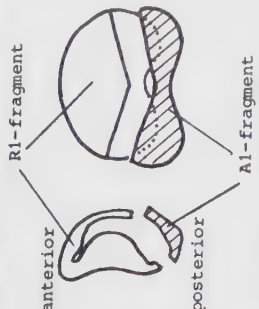
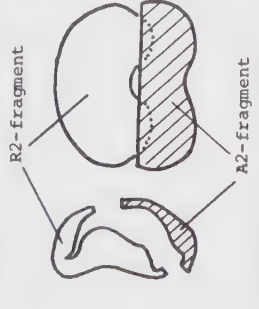
The process of reduction is less advanced in segment-8 than in segment-9 because in males, the primordium of the 8th

imaginal segment still produces a strongly reduced tergo-sternite-8 (t/s-8) which is fused to that of segment-7 (Crampton, 1942; Emmert, 1972a, b). In a female animal the primordium of the 8th imaginal segment forms the female genital apparatus (♀ Gt) and the 8th tergite (t-8).

The third level of reduction is reached in Drosophila melanogaster (Figure 27d). Here all terminal imaginal anlagen are fused into a single genital disc. At this stage of reduction the primordial cells of the 8th imaginal segment develop adult structures only in females (♀ Gt plus t-8) whereas those of the 9th segment do so only in males (♂ Gt plus GA). Nevertheless, the primordial cells of the reduced segment are still present as a "repressed" primordium (RMP/RFP) in the genital disc.

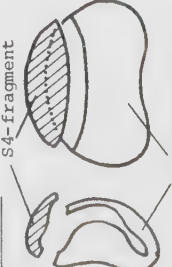
In summary, the presented phylogenetic aspects show that there is a tendency during evolution to reduce the postabdominal segments (Crampton, 1942). This reduction can be total, i.e. result in the inability of a primordium to differentiate an imaginal pattern. Since in the two sexes different segments are affected, sexual dimorphism becomes as pronounced as in Drosophila melanogaster.

Table 1 Numbers (n) and frequencies (%) of structures produced by fragments of the dorsal epithelium of the mature female genital disc subjected to immediate metamorphosis

cutting type		type and number of fragments	external genitalia	uterus, oviduct	seminal receptacle	2 spermathecae	1 spermatheca	2 parovaria	1 parovarium	dorsal anal plate complete	dorsal anal plate reduced	ventral anal plate complete	ventral anal plate reduced
series 1 (low cutting line)													
	anterior	R1:n=21 (%)	21 (100)	21 (100)	21 (100)	20 1* (100)		20 (95.3)	1 (4.7)				11 (52)
	posterior	A1:n=13 (%)	2 (15)							13 (100)		4 (31)	8 (61.5)
series 2 (high cutting line)													
	anterior	R2:n=14 (%)	14 (100)	14 (100)	14 (100)	14 (100)		14 (100)					1 (14)
	posterior	A2:n=14 (%)	6 (43)							14 (100)		12 (86)	1 (14)
* One fragment produced 3 spermathecae													

TABLES AND FIGURES

Table 1 (continuation)

cutting type	type and number of fragments	external genitalia	uterus, oviduct	seminal receptacle	2 sperma- thecae	1 sperma- theca	2 parovaria	1 parovarium	dorsal anal plate complete	dorsal anal plate reduced	ventral anal plate complete	ventral anal plate reduced
series 3 	S3:n=7 (%)				1* (14.3)	2 (28.5)		1 (14.3)				1 (14.3)
	R3:n=7 (%)	7 (100)	7 (100)	7 (100)	3 1* (57.1)	1 (14.3)	1 (14.3)		7 (100)		6 (85.7)	1 (14.3)
series 4 	S4:n=19					3 (15.7)						
	R4:n=22 (%)	22 (100)	22 (100)	22 (100)	18 1* (90.8)	1 (4.5)		9 (41)	18 (81.8)	4 (18.2)	17 (77.3)	15 (22.7)
series 5 	S5:	not transplanted										
	R5:n=9 (%)	9 (100)	9 (100)	9 (100)	9 (100)		1 (11.1)		8 (88.8)	1 (11.1)	8 (88.9)	1 (11.1)

and series C (late irradiation (120-150 hM) and 10-14 days of in vivo culture of the discs before metamorphosis).

(for abbreviations of male genital structures see index of figure 1 p 66)

clone number	time of irradiation	culture period (d)	GA	Lp	Lp*	Cl	PM	Pe	d Pa	v Pa	1 Pt	HB	Hy	Hyph	Ad	SP	ed	clone in analia	+ Gt
Series B																			
1	54*)	-							+									2 ♂ b	s
2	54	-		++	+	++							+		+				s
3	54	-				+							+						p
4	120	9	++	++	+	++												13 ♂ b	s
5	120	13	++	++		++						++	+	+				4 ♂ b	p
6	120	13	++	+	++	++						++	+	+	(+)	+			s
7	120	13	++	++		+										++	+		d
8	120	13			+	+	+	+											s
9	120	10	++	++	+	+	+	+	+		+	+	+	+	+	++	+	5 ♂ b	s
10	120	13						+				+							s
11	140	10	+			++		+	+		+	++	+	+	+	++	+		s
12	140	10				++					+			+	+	+	+		s
13	150	13									+								s
14	150	13				+						++	+					6 ♂ b	s
Series C																			

*) h, Minute time, AEL
 ++ complete structure

+ reduced structure
 (+) identification unclear

♂ b) male bristle
 ♀ b) female bristle

♂ Ct) female genital derivatives
 ♀ s) survived
 d) lost

Table 3 Sexual phenotype and size of clones in the anal plates of 35 cultured genital discs [series A }
 $\overline{Y} w; Dp (1;3)sc^{J4} + \overline{Y} M(3)1^{55} / tra$ larvae were irradiated with 2000 rads at 144 + 4 h Minute-time.
 Their genital discs were removed prior to metamorphosis and injected into 74 + 2 h wildtype larvae.

phenotype of bristles	disc number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19-35
number of $\overline{Y}$ female anal bristles	30	36	28	54	61	30	41	32	17	23	3)	44	35	35	26	38	31	18	45	
number of $\overline{Y}$ female anal bristles																				
number of $\overline{Y}$ male anal bristles	4	2	7	35			8	7	5	5	1	2	4	1	6	1	4	1	1	
total number of bristles 1)	34	38	-	-	65	38	48	37	-	-	-	50	45	39	60	42	38	-	-	

1) normal female has about 37 anal bristles

2) disc cut into sagittal halves before culture; the total number of bristles is presented in the table

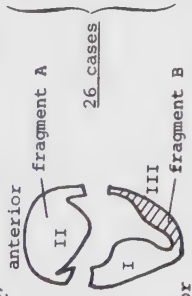
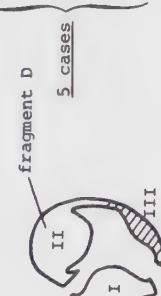
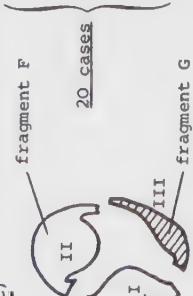
3) only one sagittal half metamorphosed

s) areas of male and female clones separated

c) areas of male and female clones in contact

Cl, Pe: anal plate clone accompanied by a clasper (Cl) or a penis (Pe) clone

Table 4 Numbers (n) and frequencies (%) of anal plates produced by fragments of the genital disc of XX; dsx /+ animals

cutting type a) 	anal plates produced by fragments A and B	n 2 (%) (7, 7)
cutting type b) 	anal plates produced only by fragment A	0
cutting type c) 	anal plates produced only by fragment B	19 59 (92, 3)
total number of tested cases: 51	anal plates produced by fragments C and D	0
	anal plates produced only by fragment C	0
	anal plates produced only by fragment D	5 (100)
	anal plates produced by fragments E and/or F and G	3 (15)
	anal plates produced only by fragments E and/or F	0
	anal plates produced only by fragment G	11 64 (85)
	frequency of cases in which only the fragment containing region III (B, D, G) produced anal plates: 46/51 (90%)	

1) in 5 cases only fragment A survived and did not produce anal plates

2) 6 times either fragment E or F died, but amount of produced anal plates by fragment G was practically normal

FIGURES

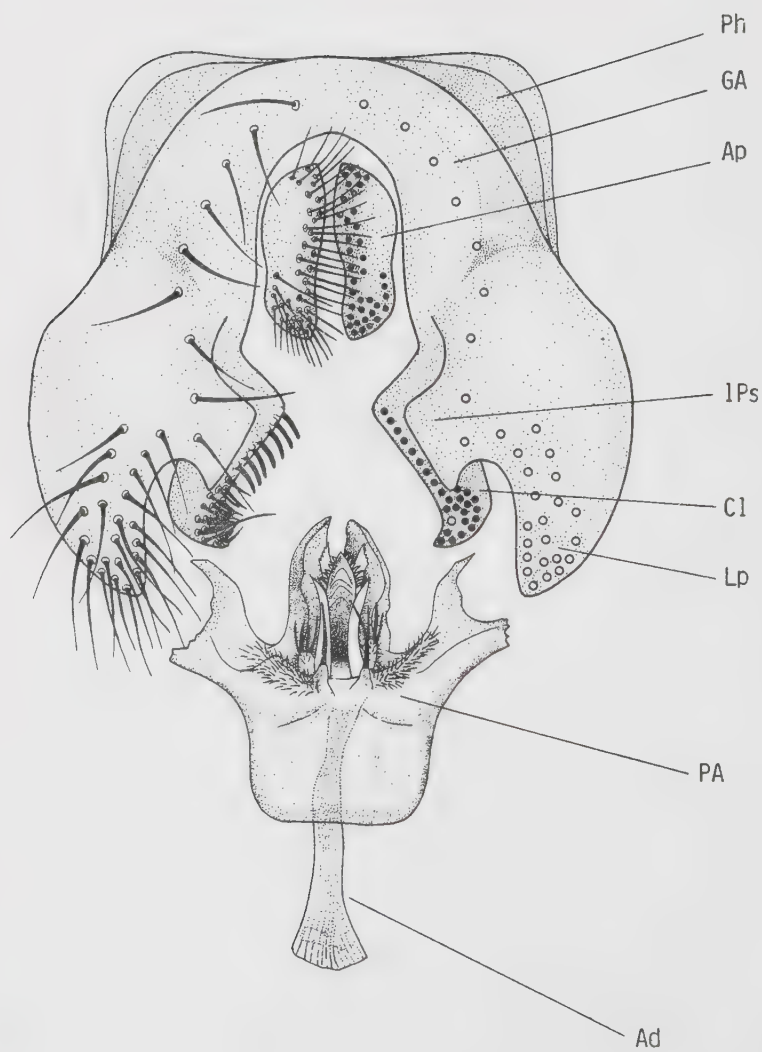
Figure 1aMorphology of the external male terminalia:

Figure 1b

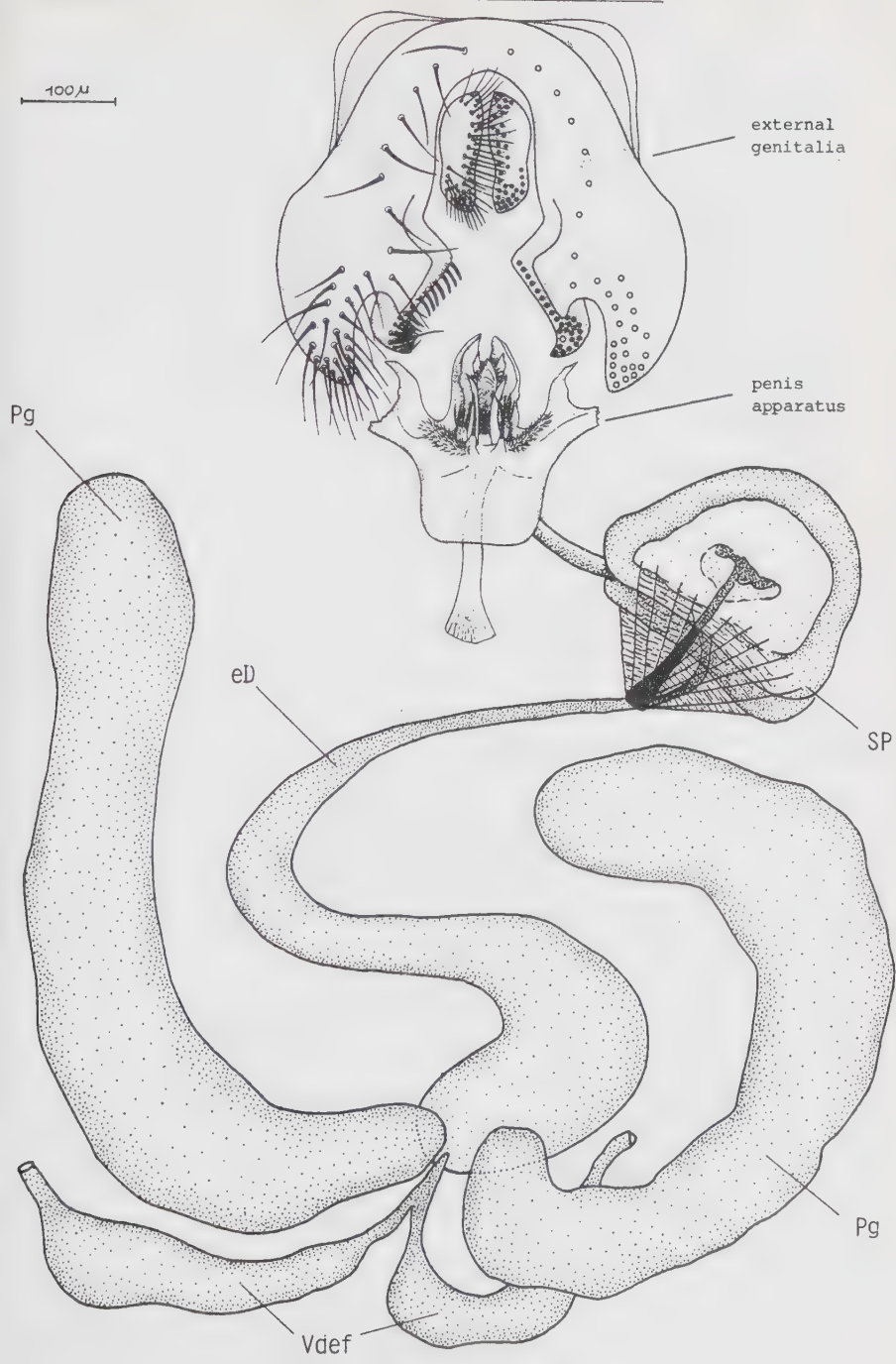
Derivatives of the male genital disc:

Figure 1 Index: (male terminalia)

Ad:	apodeme	additional abbreviations for Table 2:	
Ap:	anal plate		
Cl:	clasper	dPa:	dorsal paramere
eD:	ejaculatory duct	HB:	hypandrial bristle
GA:	genital arch	Hy:	hypandrium
Lp:	lateral plate	HyPh:	hypandrial phragma
lPs:	lateral processus of the genital arch	lPt:	lateral protuberance
PA:	penis apparatus	Pe:	penis
Pg:	paragonium	PM:	penis mantle
Ph:	phragma of the genital arch	vPa:	ventral paramere
SP:	sperm pump		
Vdef:	vas deferens		

(for detailed illustration of the penis apparatus see figure 23, p 105)

Figure 2 Index: (female terminalia)

Spt:	spermatheca
R:	seminal receptacle
Od:	oviduct
U:	uterus
Vu:	vulva (d: dorsal, v: ventral)
s:	sensilla trichodea
thb:	thorn bristle
lb:	long bristle
Vp:	vaginal plate
T-8:	tergite-8
Pov:	parovarium
dAp:	dorsal anal plate
vAp:	ventral anal plate

Figure 2

Derivatives of the female genital disc:

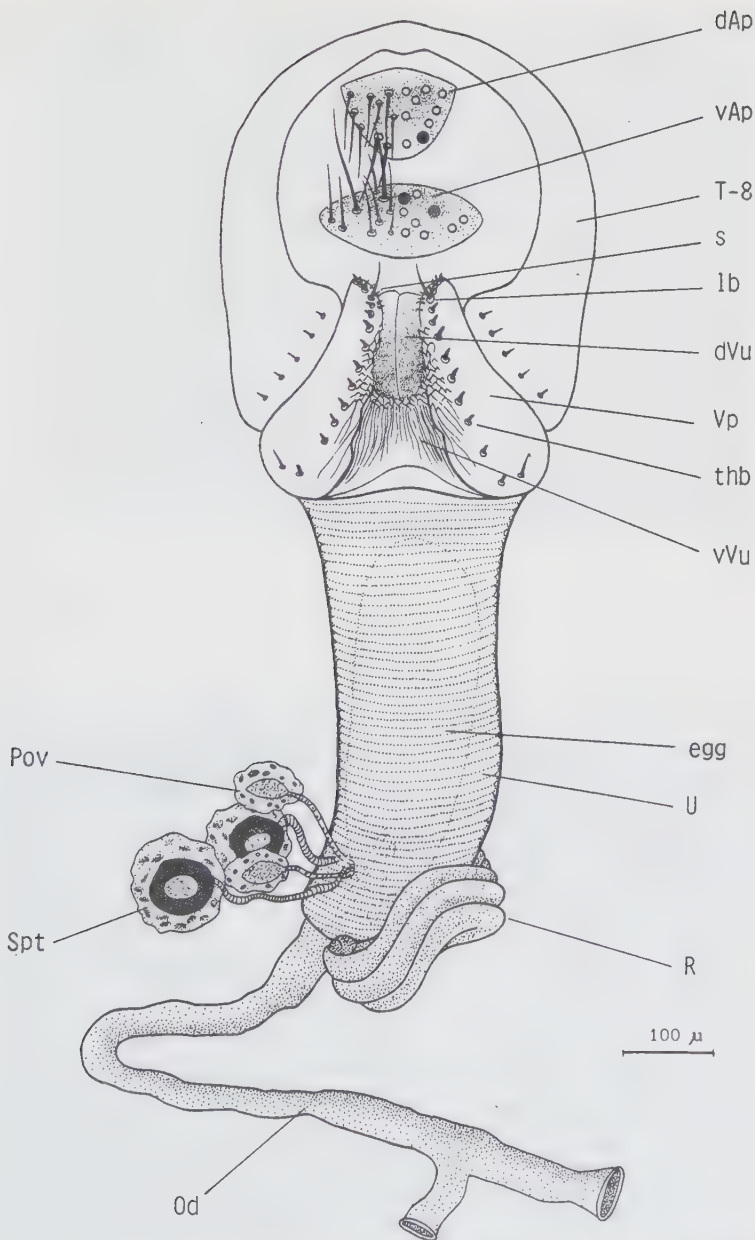


Figure 3 The terminalia of XX; dsx^D/+ animals:

Abbreviations:

male genital elements:

female genital elements:

GA: genital arch	Spt: spermatheca
Lp: lateral plate	R: seminal receptacle
Cl: clasper	Od: oviduct
SP: sperm pump	U: uterus
eD: ejaculatory duct	Vp: vaginal plate
Pg: paragonium	T-8: tergite-8
HB: hypandrial bristle	
PM: penis mantle	Pdu: penis duplication
Pe: penis	Ap: anal plate
Vdef: vas deferens	
ext: external male genitalia	

Figure 4 Morphology of the male genital disc:

The "silent region" (SR), which does not contribute to the formation of the male terminalia, is hatched in figure 4b. The "silent region" is believed to be the "repressed" female genital primordium (RFP) in the male disc.

Abbreviations:

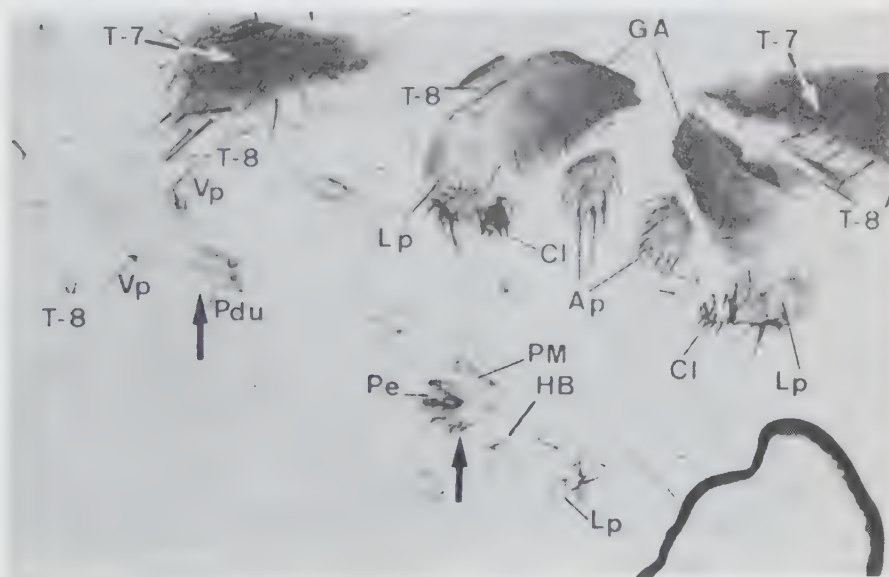
a: anterior	aB: anterior bulbus
p: posterior	LL: lateral lobe
l: left	st: stalk
r: right	e/e' : plane of parasagittal cut
dS: dorsal sheath	Apl/r: left/right anal plate
vS: ventral sheath	SR: "silent region" (=RFP)
RFP: "repressed" female genital primordium (=SR)	

Photographs from Figure 4 c), d) and e) were taken by P. Ehrensperger

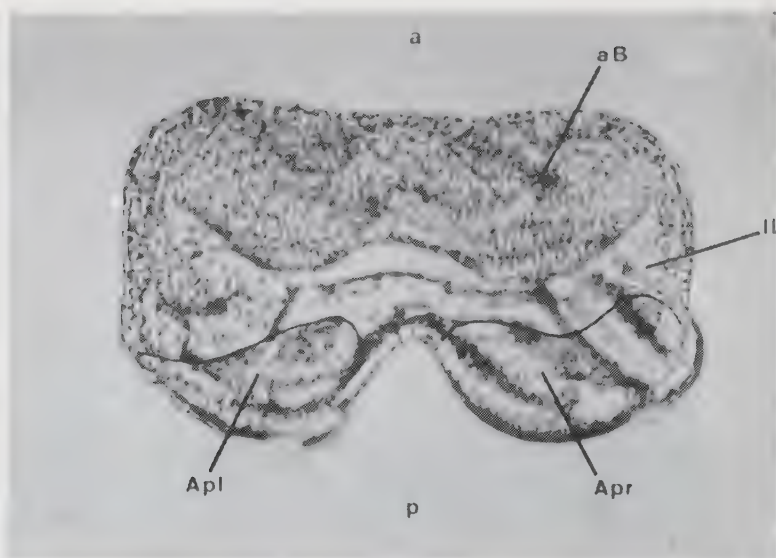
Figure 3 The terminalia of XX;dsx^D/+ animals:



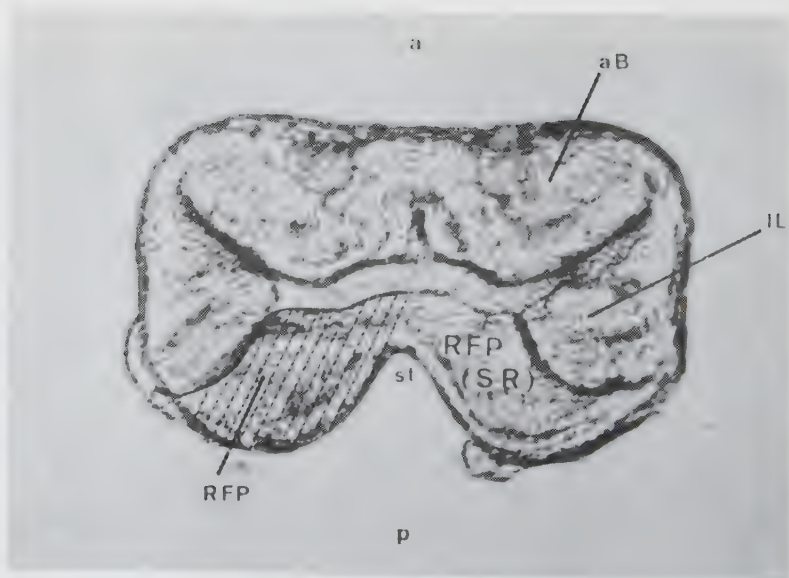
a) Derivatives of a transplanted dsx^D-genital disc



b) External terminalia of a vv; dsx^D/+ fly

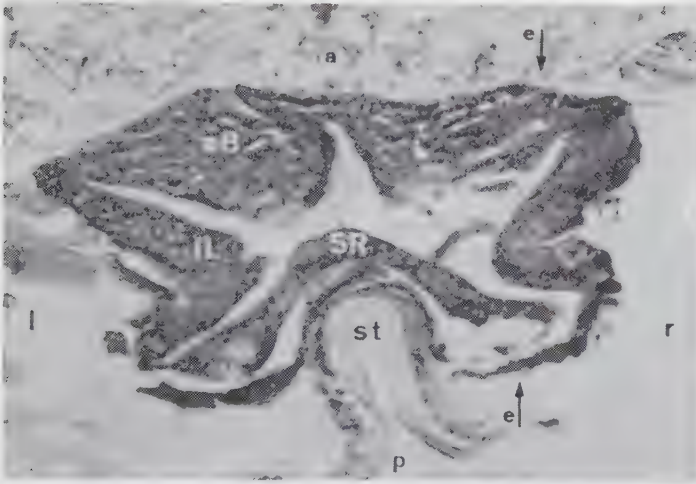


a) dorsal view

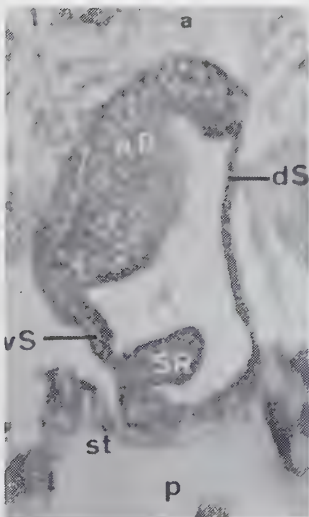


b) ventral view

Figure 4 Morphology of the male genital disc (continuation):



c) Cross-section



d) sagittal section



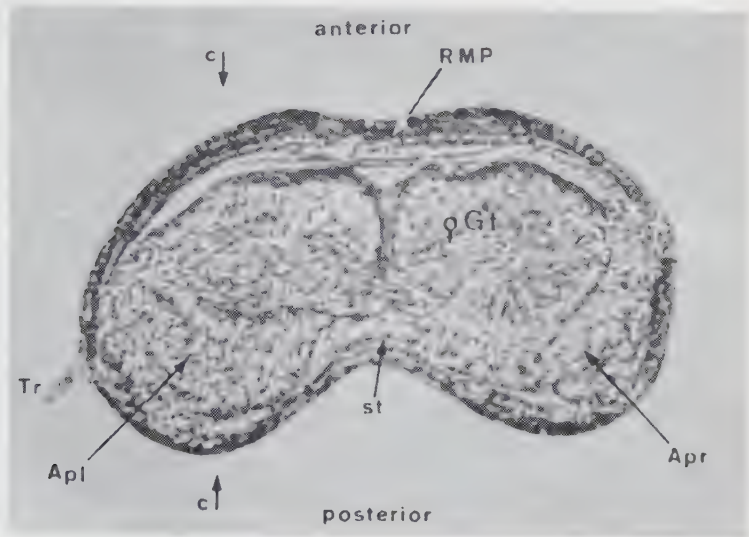
e) parasagittal section

Figure 5

Morphology of the female genital disc. a) dorsal view, b) sagittal section, c) parasagittal section at the level which is indicated with $c\downarrow/c\uparrow$ in Figure 5a.

a:	anterior
p:	posterior
st:	stalk
♀ Gt:	female genitalia
RMP:	"repressed" male primordium
Apr/Apl:	right/left anal plate
Tr:	trachea
$c\downarrow$:	plane of parasagittal section
Gi:	internal genitalia
Ge:	external genitalia
vS:	ventral sheath
dS:	dorsal sheath
Pov:	parovaria
Ap:	anal plate

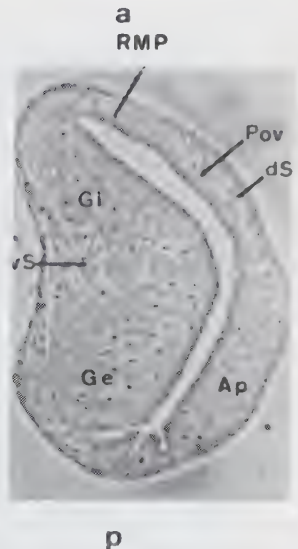
Figure 5 Morphology of the female genital disc



a) dorsal view



b) sagittal section



c) parasagittal section

Figure 6

Morphology of the dsx^D -genital disc. a) ventral view, b) sagittal section, c) parasagittal section at the level which is indicated by c_1/c_1^* in Figure 6a.

a:	anterior
p:	posterior
♂ Gt:	male genitalia
♀ Gt:	female genitalia
aB:	anterior bulbus
lL:	lateral lobe
Tr:	trachea
st:	stalk
I,II,III:	major regions of the disc
vS:	ventral sheath
dS:	dorsal sheath
AP:	anal plate

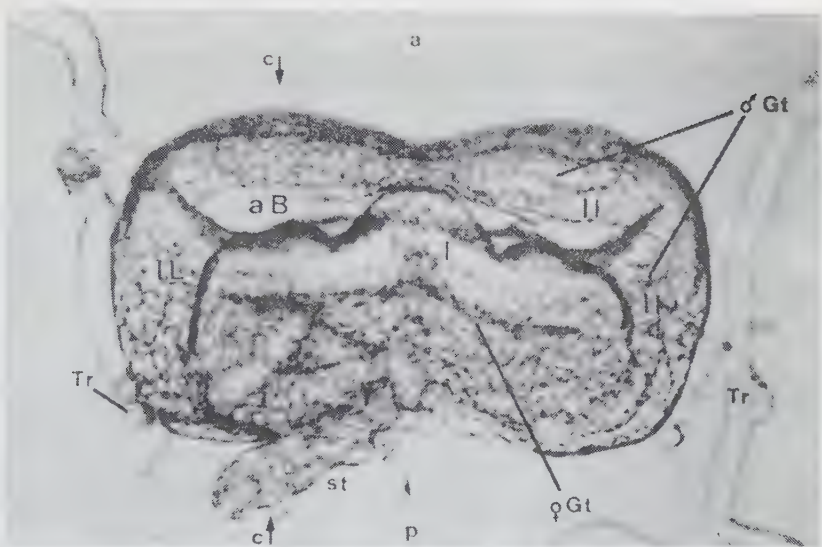
Figure 7

Continuous series of sections through a sagittal half of a dsx^D -genital disc. (a) lateral edge, (b) parasagittal section cutting through the male lateral lobe (lL), (c) parasagittal section through a level at about 30% of the left-right extension of the sagittal half, (d) sagittal section.

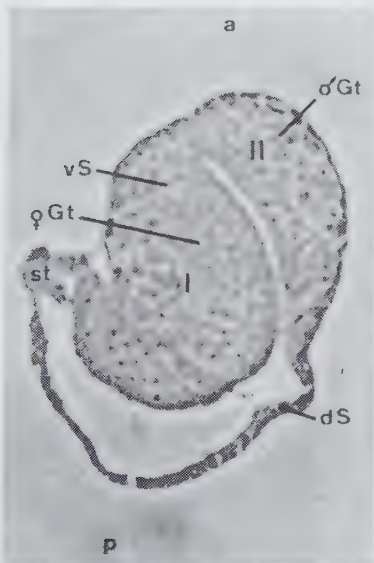
vS:	ventral sheath
dS:	dorsal sheath
a:	anterior
p:	posterior

regions III, II and I are the anal, male and female genital primordia, respectively.

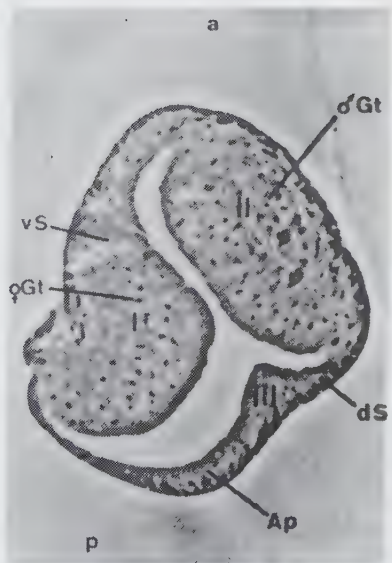
Figure 6 Morphology of the dsx^D-genital disc



a) ventral view



b) sagittal section



c) parasagittal section

Figure 7 Continuous series of parasagittal sections through a sagittal half of a dsx^D -genital disc (for abbreviations see p 74)

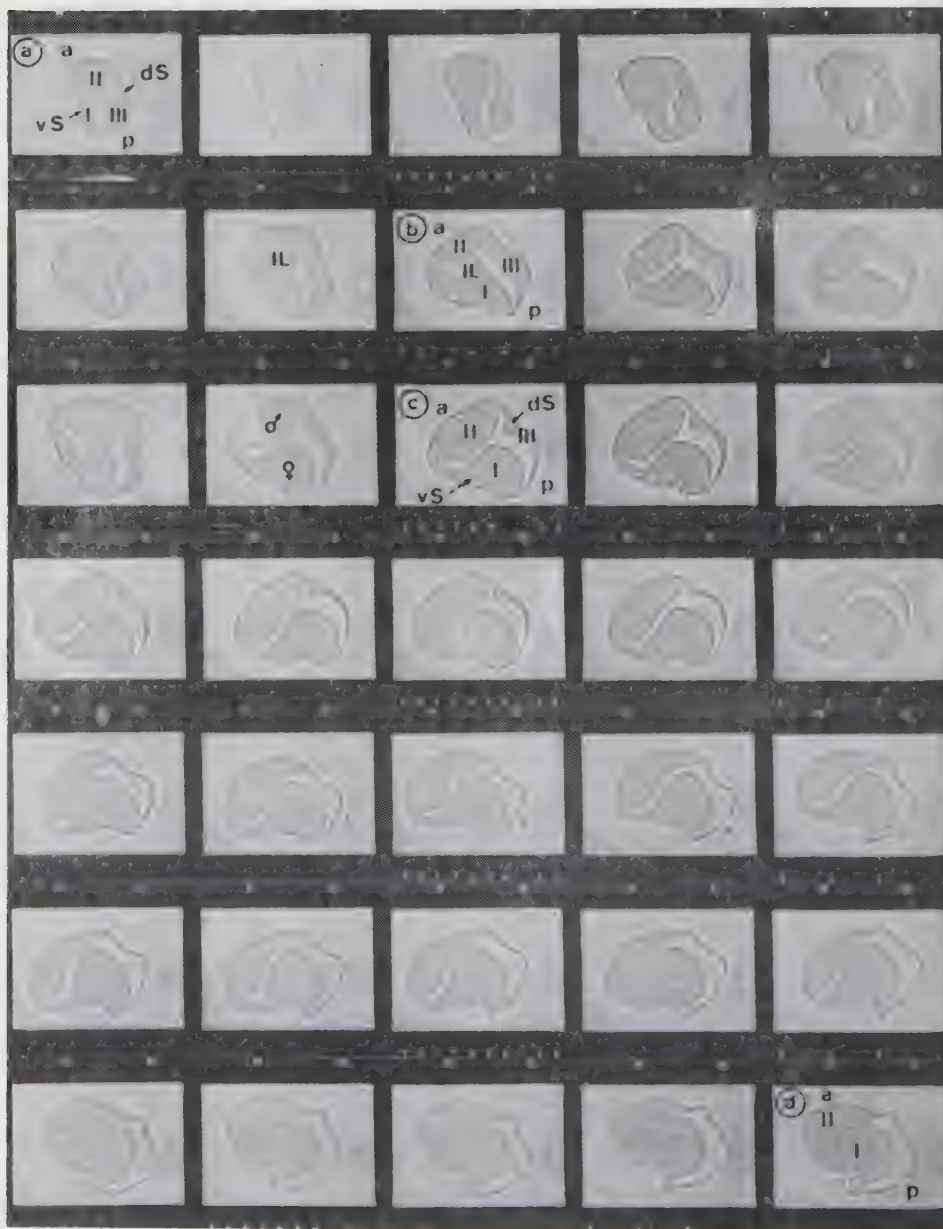
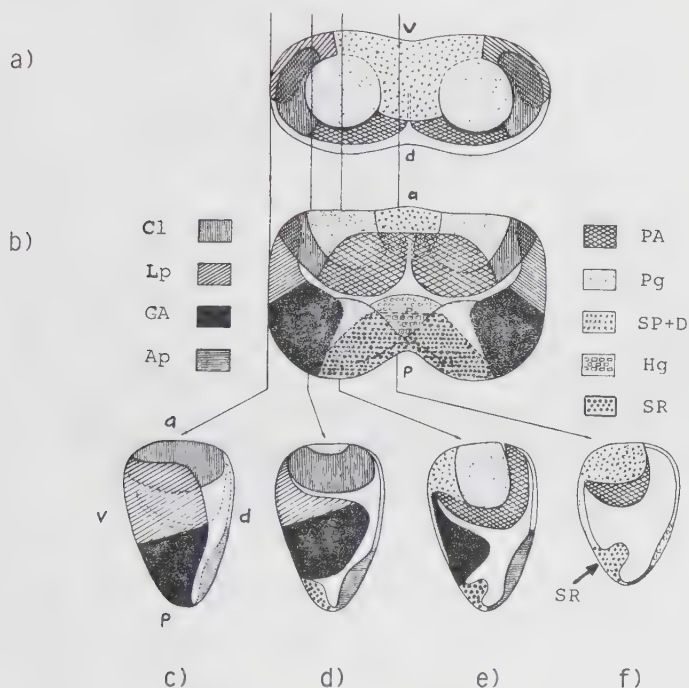


Figure 8 Three-dimensional anlageplan of the male genital disc: *)

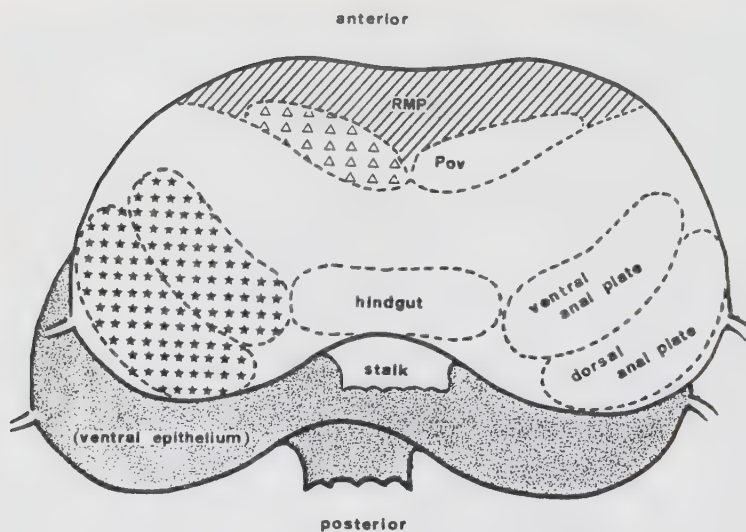


a) anterior view; b) dorsal view (anterior to the top); c) lateral view from left; d) e) f) parasagittal sections (ventral to the left).

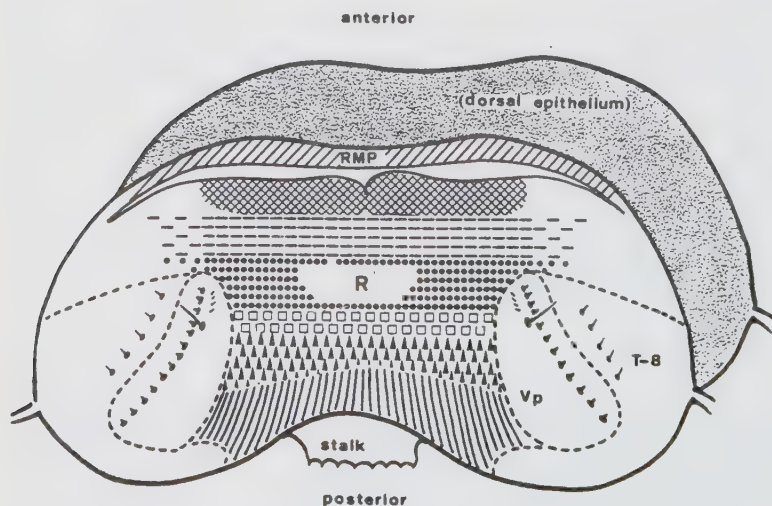
Abbreviations: a: anterior; p: posterior; d: dorsal; v: ventral; Ap: anal plates; Hg: hind gut; Cl: clasper; GA: genital arch; Lp: lateral plate; PA: penis apparatus; Pg: paragonium (this area also includes the anlage of the vas deferens); SP+D: sperm pump and ejaculatory duct; SR: "silent region", which does not form adult structures [the "silent region" turned out to be the "repressed" female genital primordium (see p 47)].

*) modified after P. Ehrensperger (1972)

Figure 9 Three-dimensional anlageplan of the female genital disc:



a) Derivatives of the dorsal epithelium



b) Derivatives of the ventral epithelium *)

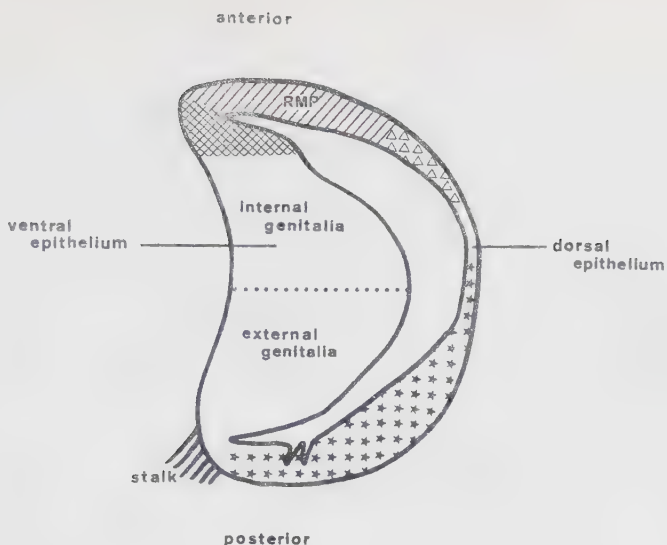


Figure 9c) parasagittal section

Legend:

△△△△	parovarium (Pov)
△△△△	
****	anal plate

XXXX	spermatheca
----	oviduct

.....	uterus
.....	
□□□□	vagina
□□□□	
▲▲▲▲	dorsal vulva
▲▲▲▲	
////	ventral vulva
////	
T-8	tergite-8
	tergite-8 bristles
Vp	vaginal plate
▲▲▲	thorn bristles
—	long bristle
///	sensilla trichodea
R	seminal receptacle
Tr	trachea
RMP	"repressed" male genital primordium (= "seilent region")

Figure 10

Organization of the genital disc. a) male disc, modified after Ehrensperger (1972), b) ^Ddsx-disc, c) female disc. The left drawings show the ventral view of the disc whereas the right ones were made after parasagittal sections of the disc. The plane of the parasagittal section is indicated with arrows (c/c) in Figure 10a.

♂ Gt:	male genitalia
♀ Gt:	female genitalia
An:	analia
RFP:	"repressed" female genital primordium
RMP:	"repressed" male genital primordium
st:	stalk
Gi:	internal genitalia
Lp:	lateral plate
GA:	genital arch
T-8:	tergite-8
Vp:	vaginal plate

*) The silent regions do not produce recognizable adult structures.

Figure 10 Organization of the genital disc:

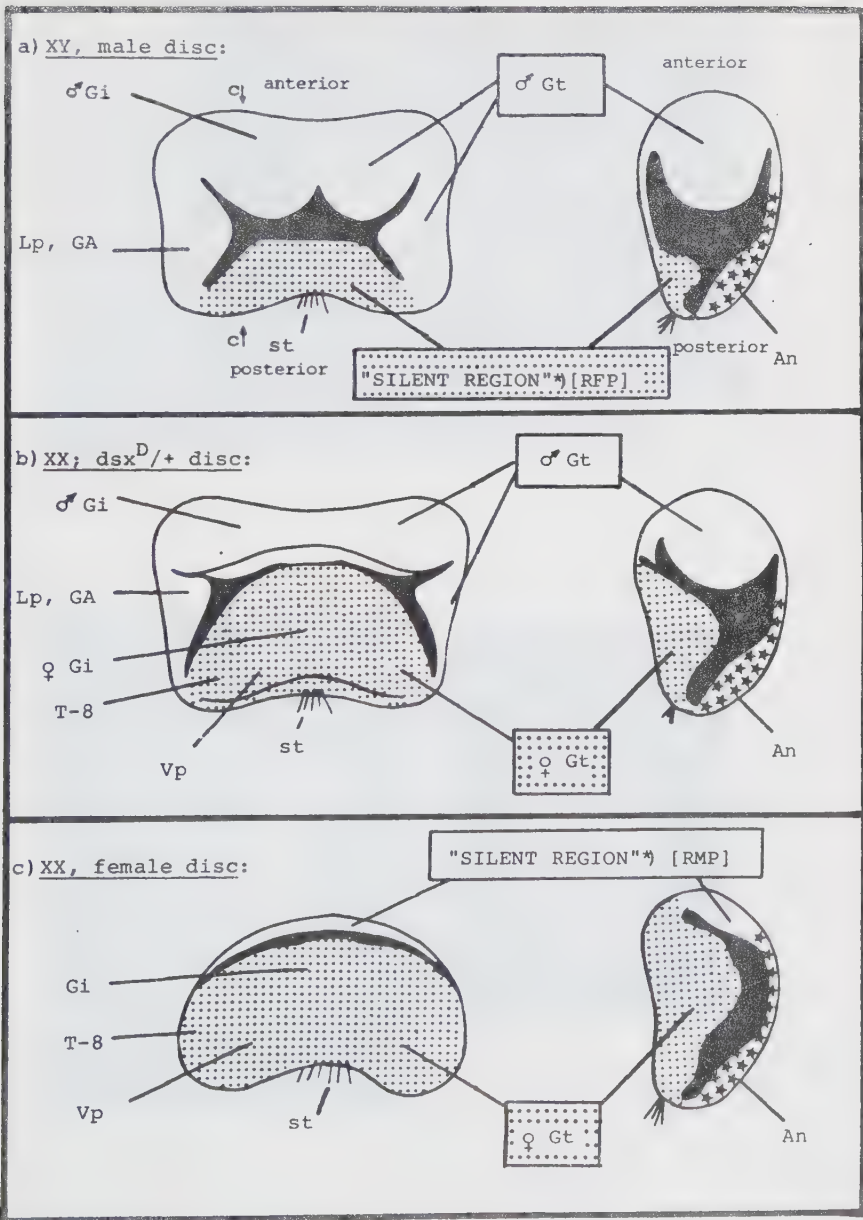
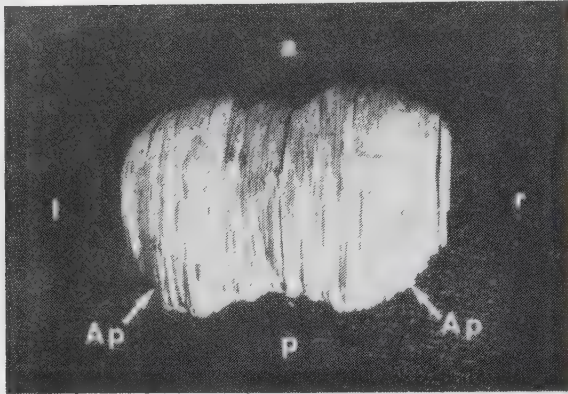
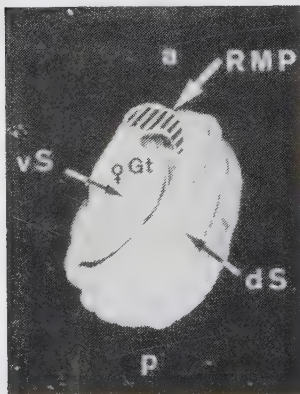


Figure 11 Reconstruction of the female genital disc:



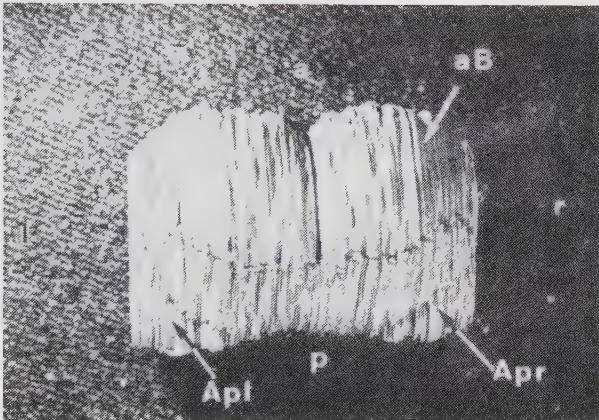
dorsal view



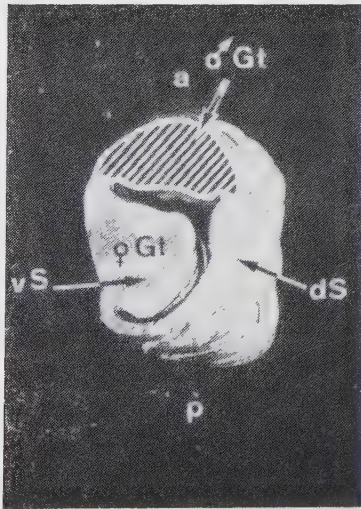
sagittal half

- ♀ Gt: female genitalia
- RMP: "repressed" male genital primordium
- a: anterior
- p: posterior
- l: left
- r: right
- vS: ventral sheath
- dS: dorsal seath
- Ap: anal plate

Figure 12 Reconstruction of the dsx^D-genital disc:



dorsal view

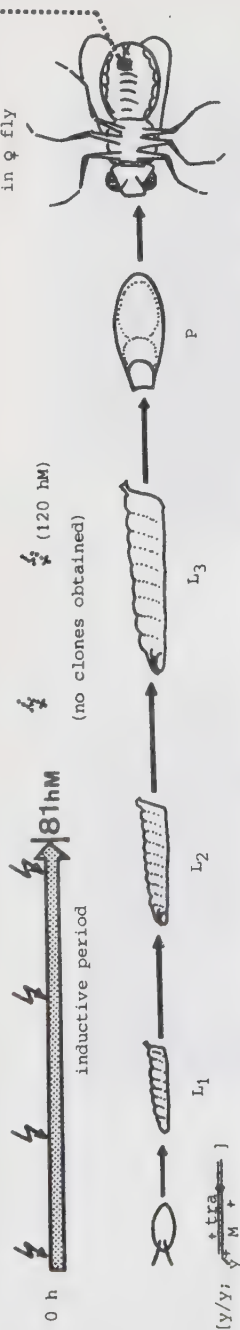


sagittal half

- ♂ Gt: male genitalia
- ♀ Gt: female genitalia
- a: anterior
- p: posterior
- l: left
- r: right
- Apl/r: left/right anal plate
- aB: anterior bulbus
- vS: ventral sheath
- dS: dorsal sheath

Figure 13 Period during which the formation of male genital structures can be induced in the female genital disc

a) in situ: (after Wieschaus and Nöthiger, personal communication)



b) with additional in vivo culture of the irradiated disc (Series A):

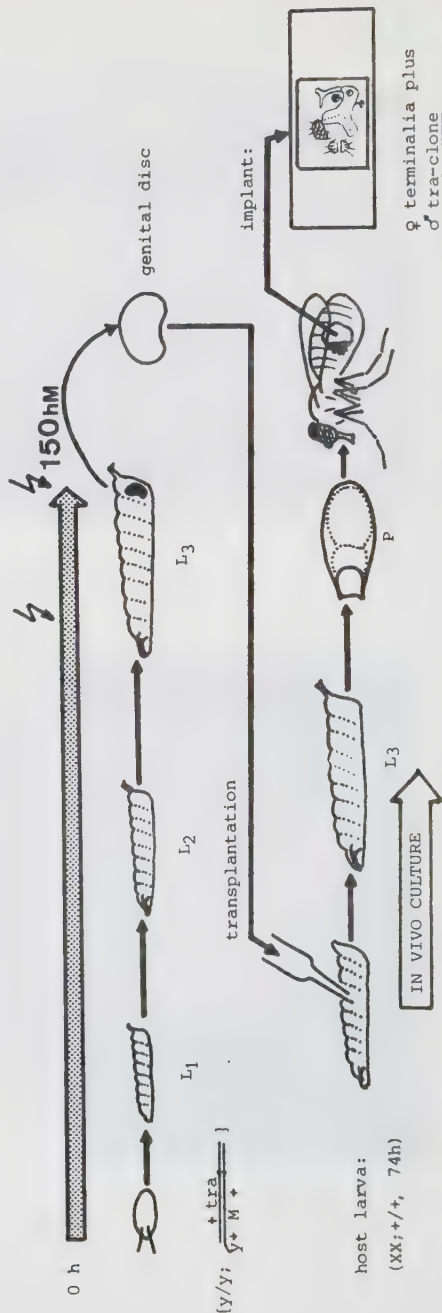


Figure 14 Experimental procedure for the localization of early induced male clones in the female disc (Series B)

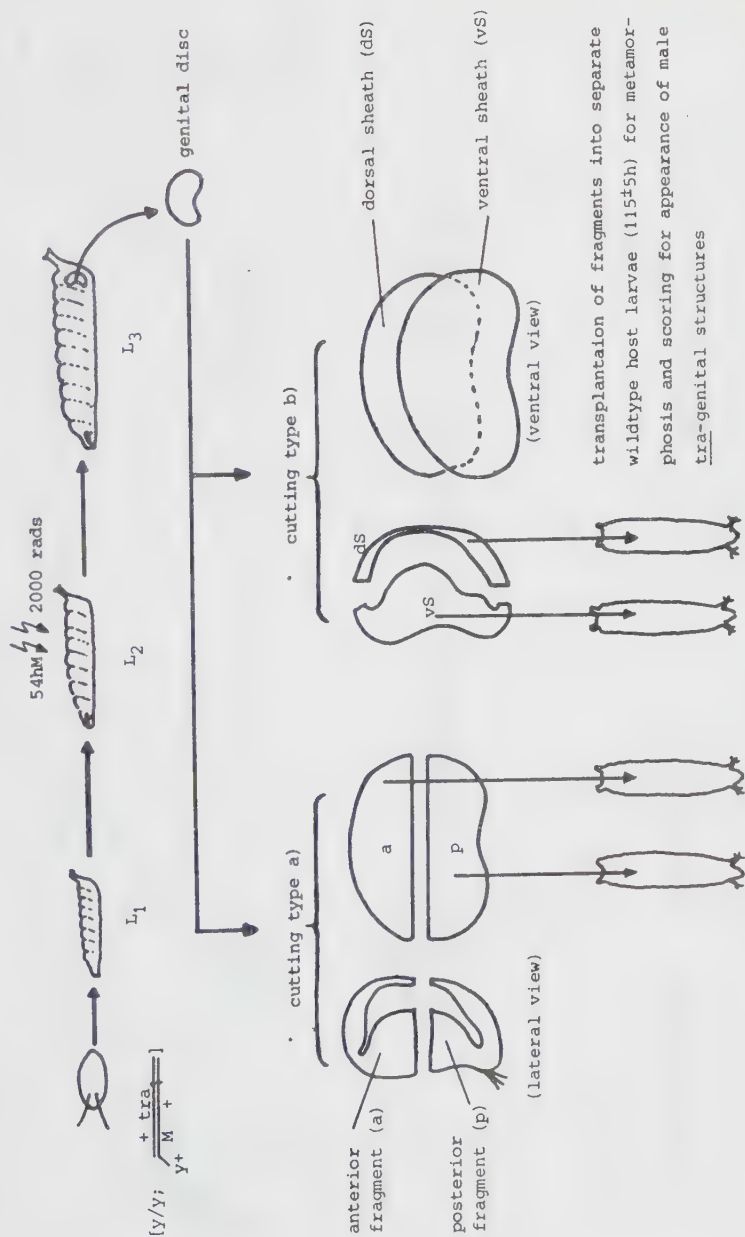


Figure 15 Experimental procedure for the localization of late induced male genital clones in the female disc (Series C)

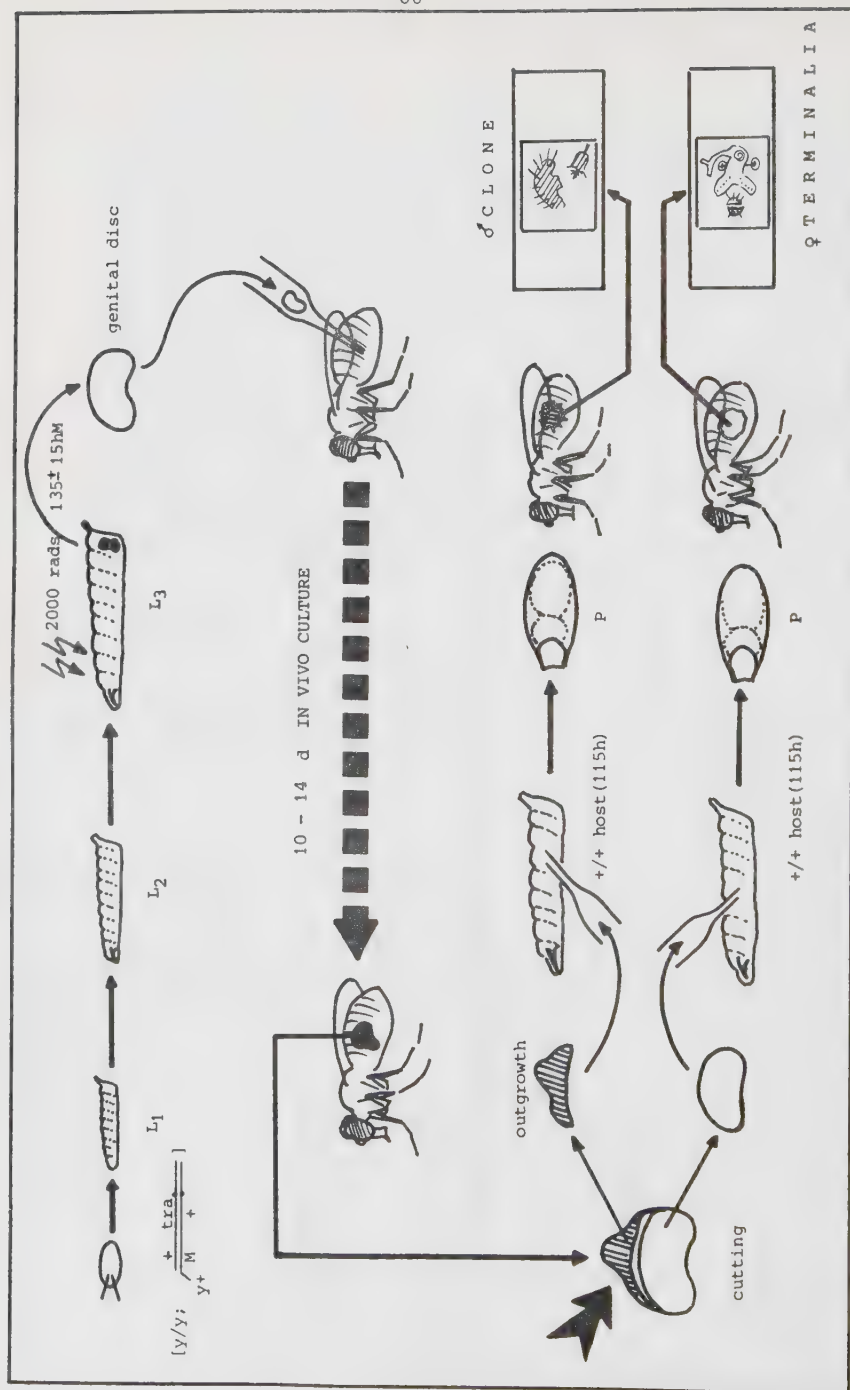


Figure 16

Morphology of clone No. 12. a) cultured disc, b) and c) photographs and drawings of the derivatives of the metamorphosed outgrowth and the rest of the disc.

♀ Gt: female genitalia
 Apl/r: left/right anal plate
 Ov: ovary
 Tr: trachea

male genital elements:

GA: genital arch
 Lp: lateral plate
 Cl: clasper
 SP: sperm pump
 eD: ejaculatory duct
 Pg: paragonium
 Ad: apodeme
 lPt: lateral protuberance
 HB: hypandrial bristle
 Hy: hypandrium
 HH: hypandrial hairs
 vPa: ventral paramere
 dPa: dorsal paramere
 PM: penis mantle
 Pe: penis
 HyPh: hypandrial phragma
 Vdef: vas deferens

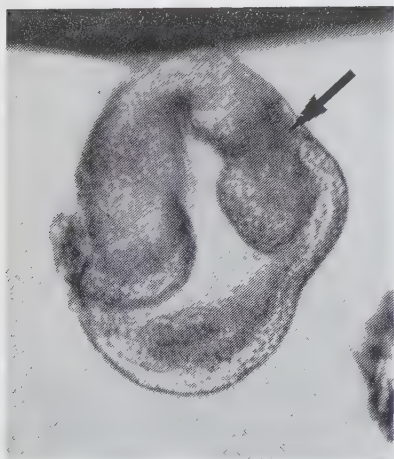
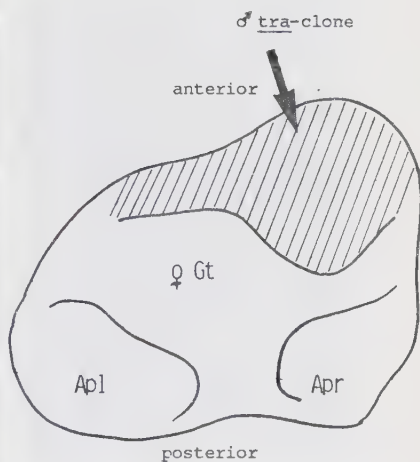
female genital elements:

Spt: spermatheca
 R: seminal receptacle
 Od: oviduct
 U: uterus
 Vu: vulva
 s: sensilla trichodea
 thb: thorn bristle
 lb: long bristle
 Vp: vaginal plate
 T-8: tergite-8
 Pov: parovarium

Figure 16a Morphology of clone No 12 (Cultured disc)



dorsal view



lateral view

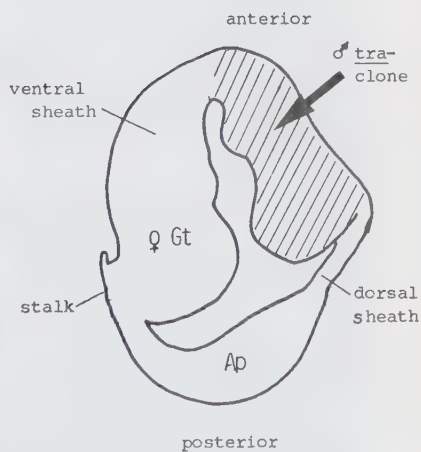
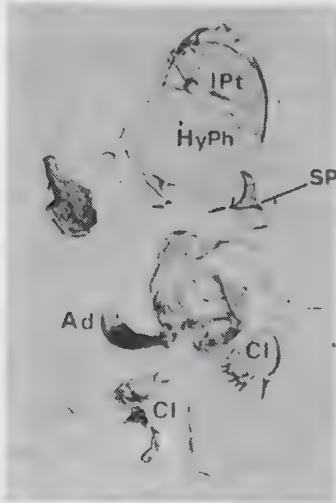
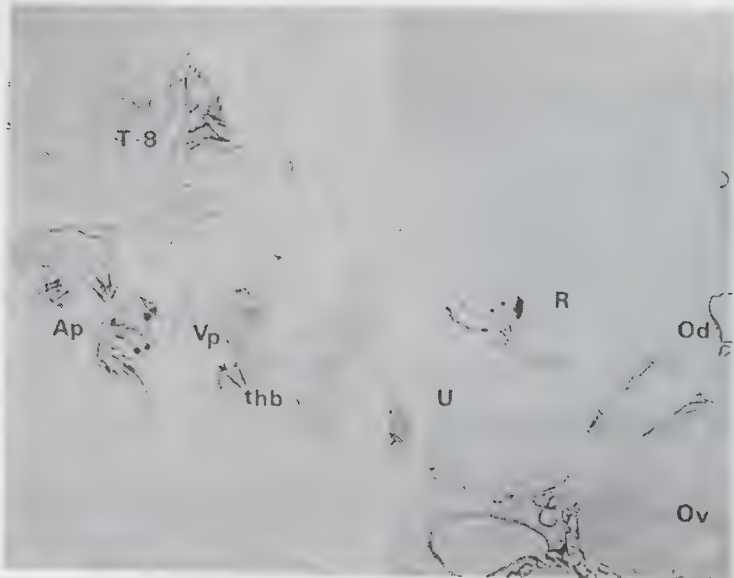


Figure 16b Derivatives of clone No 12

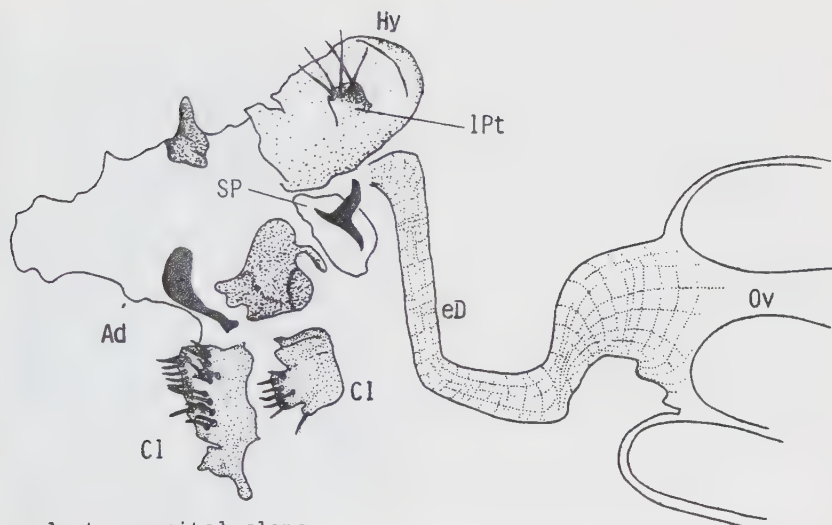


male tra-genital clone

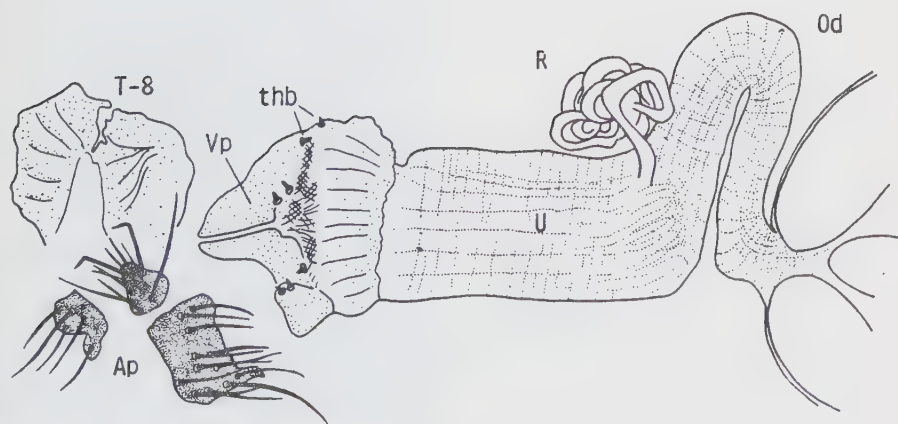


female terminalia

Figure 16c Derivatives of clone No 12



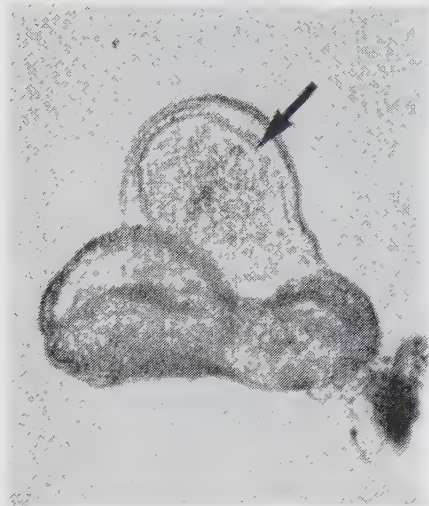
male tra-genital clone



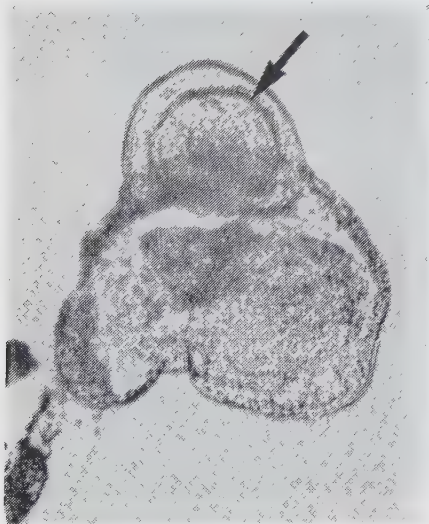
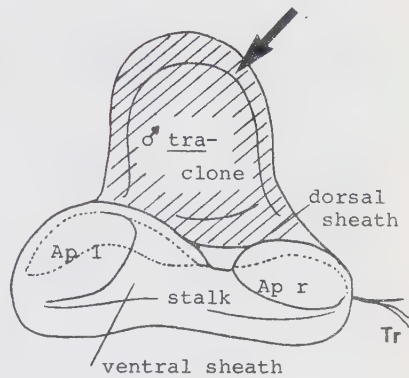
female terminalia

Figure 17 Morphology of clone No 9

a) Cultured genital disc (for abbreviations see fig. 16)



posterior view



anterior view

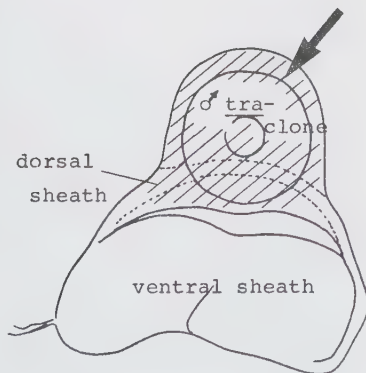
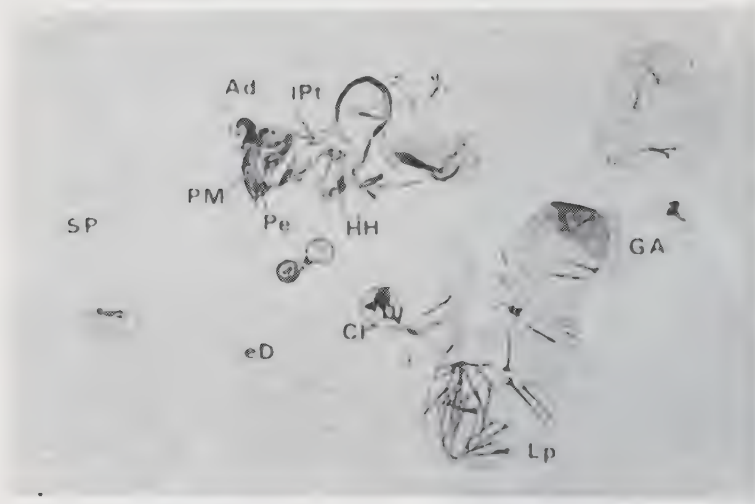


Figure 17b Derivatives of clone No 9

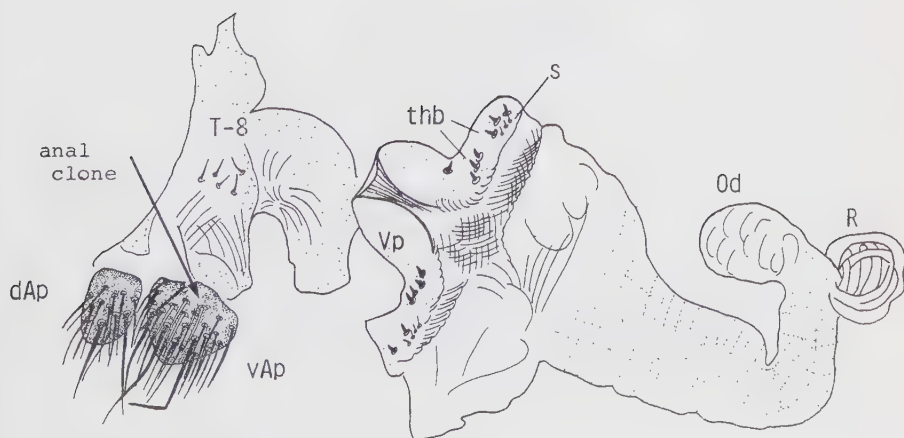
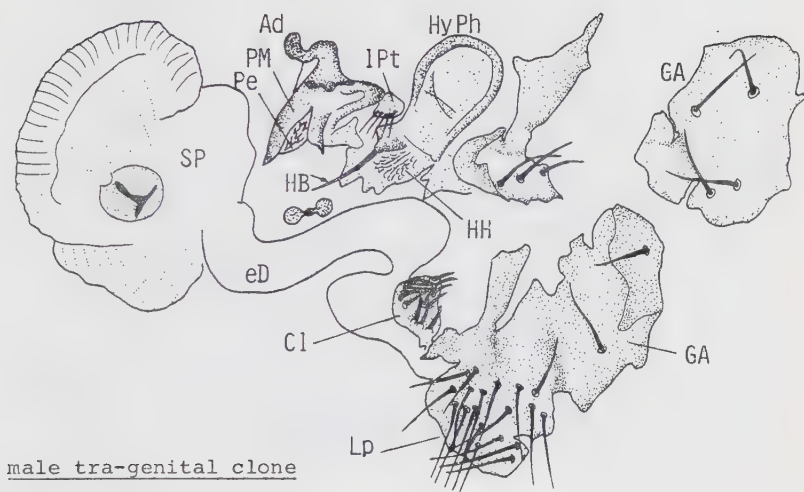


male tra-genital clone



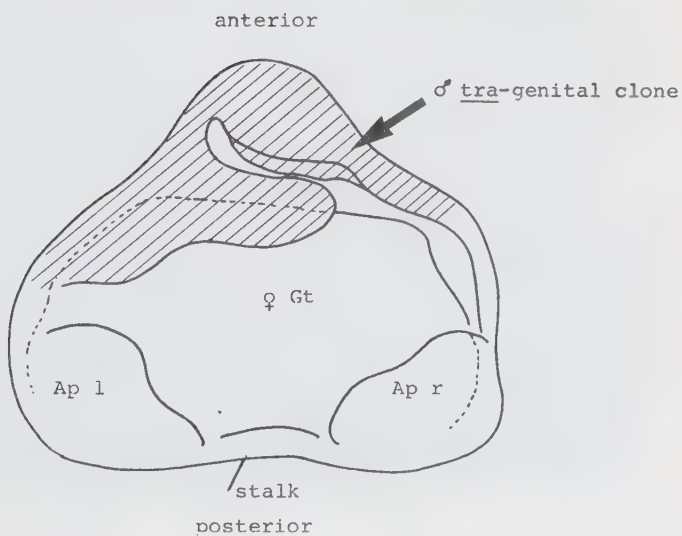
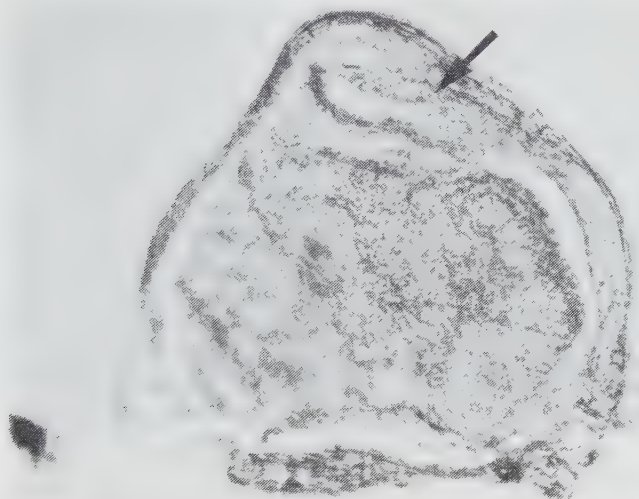
female terminalia

Figure 17c Derivatives of clone No 9



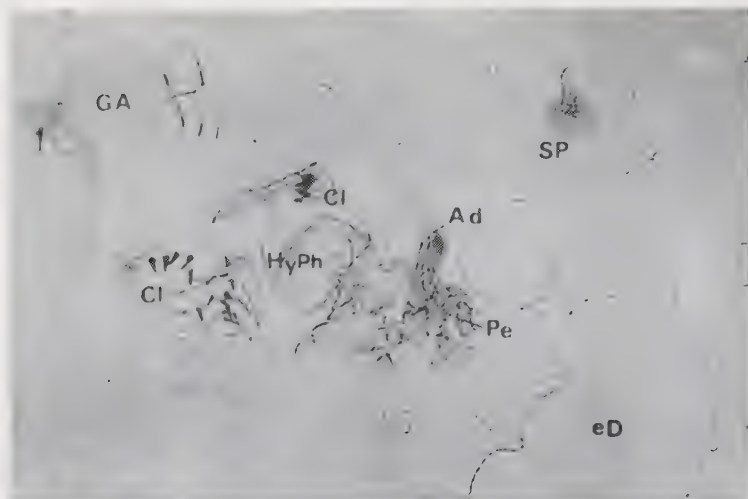
female terminalia

Figure 18a Morphology of clone No 11 (cultured genital disc)
 (for abbreviations see index of figure 16)

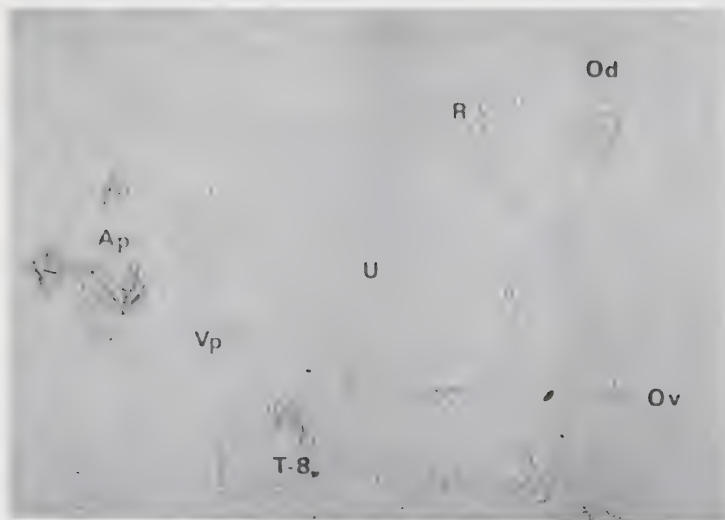


posterior view

Figure 18b Derivatives of clone No 11



male tra-genital clone



female terminalia

Figure 18c Derivatives of clone No 11

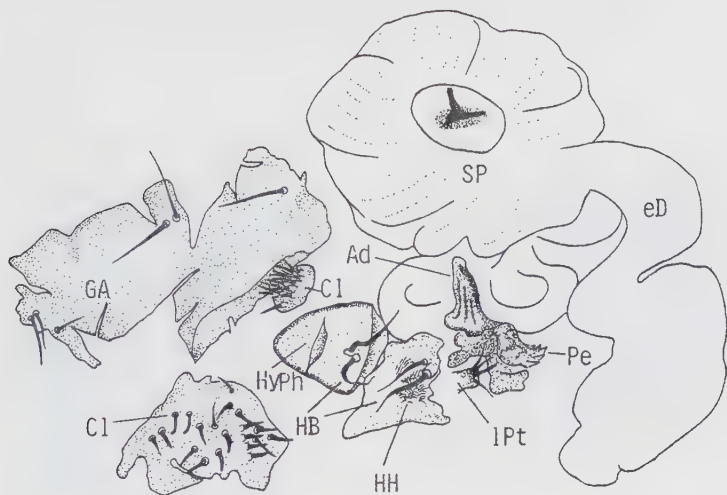
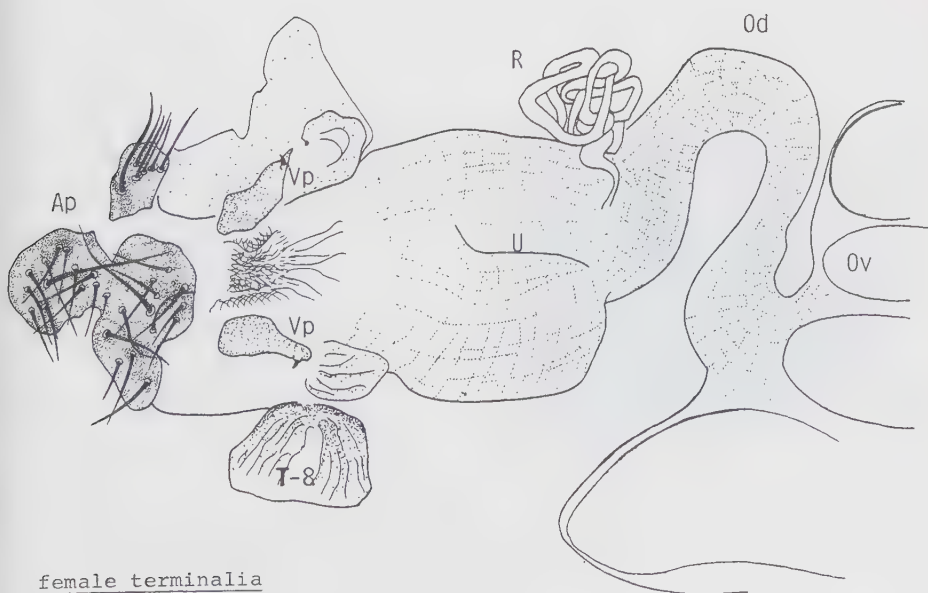
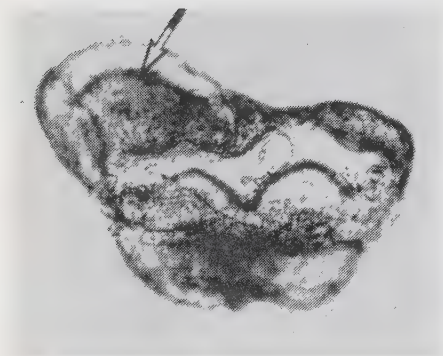
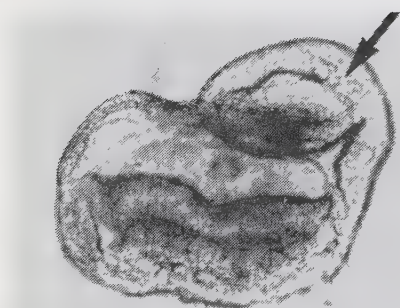
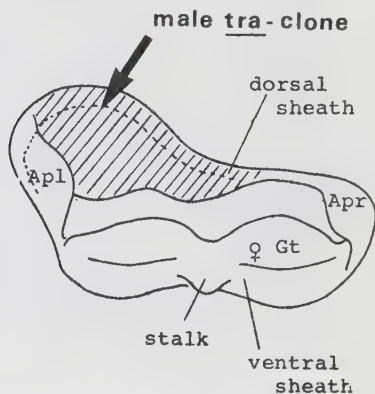
male tra-genital clonefemale terminalia

Figure 19a Morphology of clone No 5 (cultured genital disc)
 (for abbreviations see index of figure 16)



posterior view



anterior view

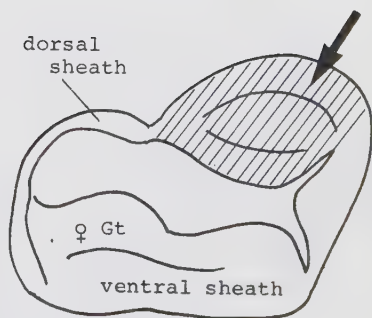
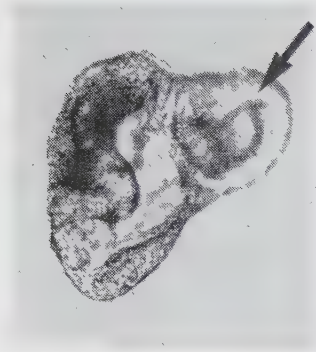
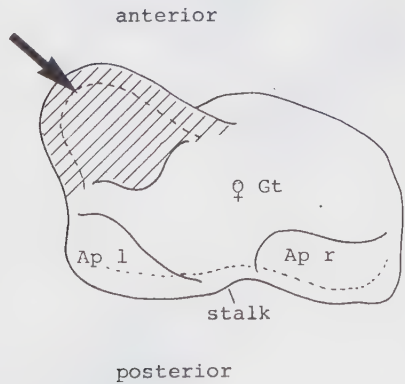
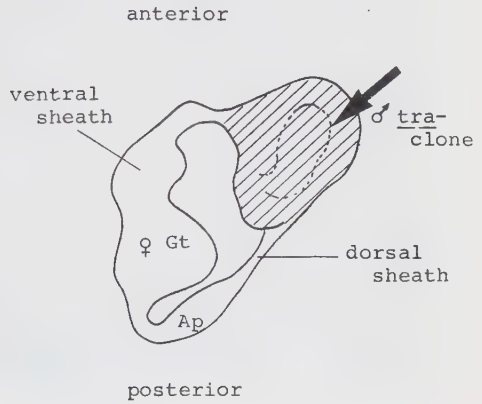


Figure 19a Cultured genital disc (continuation)
 (for abbreviations see index of figure 16)

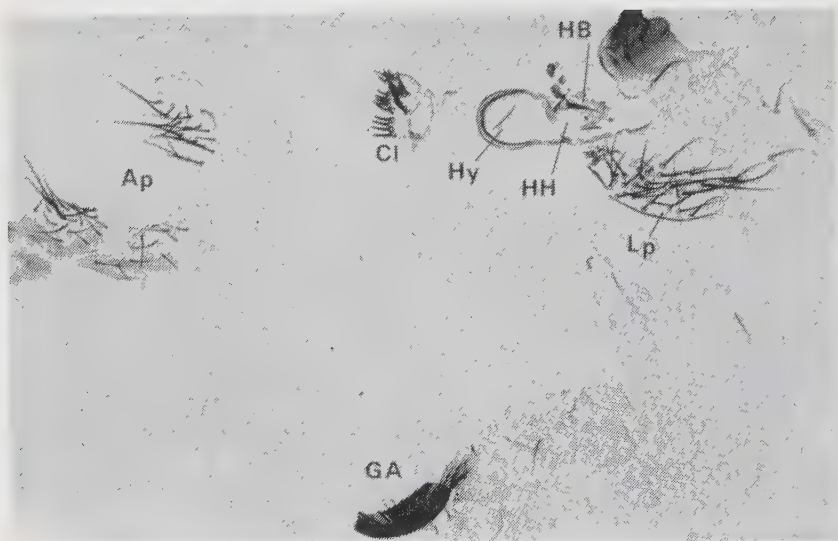


lateral view

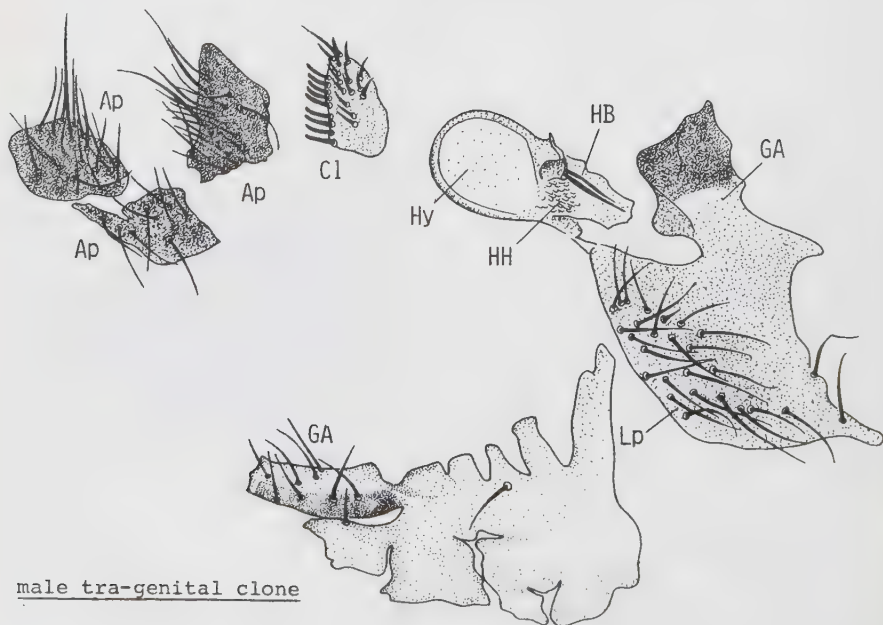


posterior view

Figure 19b Derivatives of clone No 5:

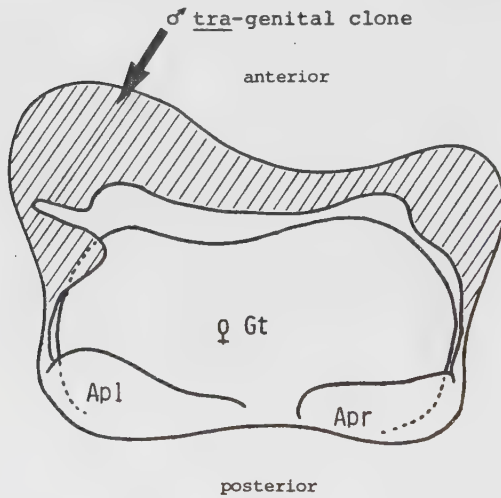


male tra-genital clone



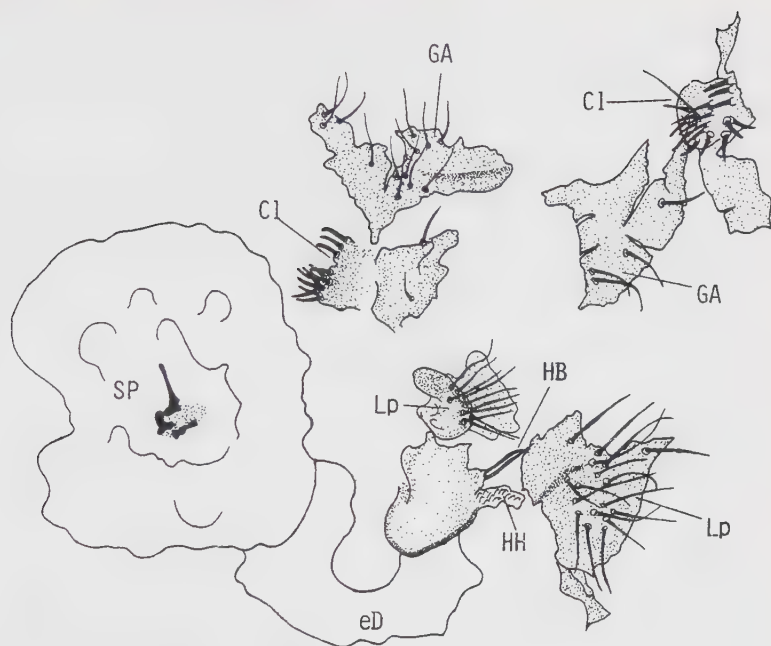
male tra-genital clone

Figure 20 Morphology of clone No 6 (cultured disc):
(for abbreviations see index of figure 16)

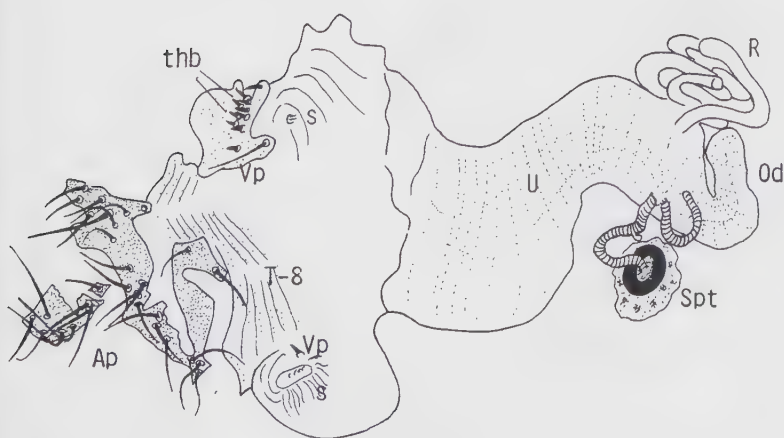


dorsal view

Figure 20 Derivatives of clone No 6



male tra-genital clone



female terminalia

Figure 21

Number of structures produced by fragments I and II of the dsx^D-disc

In order to reveal the developmental fate of regions I and II, these fragments were injected into separate mature host larvae. After metamorphosis, the implants were analyzed for the presence of male and female genital structures.

- Dotted area: derivatives of fragment I
- Hatched area: derivatives of fragment II

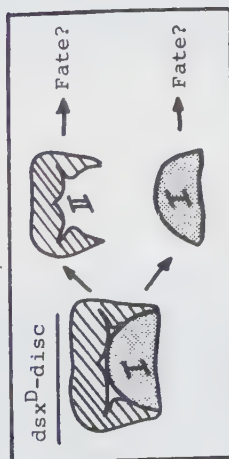
male genital elements:

GA: genital arch
 Lp: lateral plate
 Cl: clasper
 SP: sperm pump
 eD: ejaculatory duct
 Pg: paragonium
 Ad: apodeme
 lpt: lateral protuberance
 HB: hypandrial bristle
 Hy: hypandrium
 vPa: ventral paramere
 dPa: dorsal paramere
 PM: penis mantle
 Pe: penis
 HyPh: hypandrial phragma

female genital elements:

Spt: spermatheca
 R: seminal receptacle
 Od: oviduct
 U: uterus
 Vu: vulva
 s: sensilla trichodea
 thb: thorn bristle
 lb: long bristle
 Vp: vaginal plate
 T-8: tergite-8
 Ap: anal plate

maximum of 60 scored fragments II



(Abbreviations see index)

maximum of 49 scored fragments I

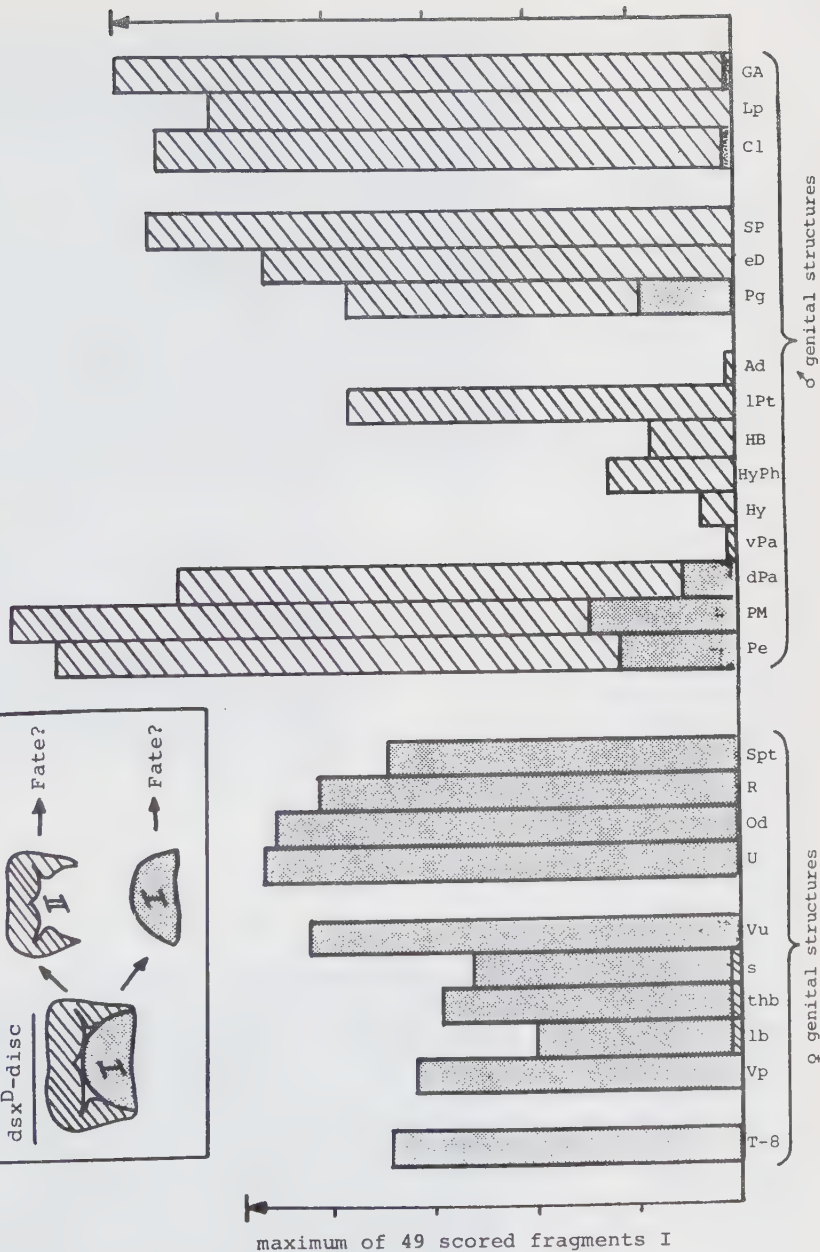
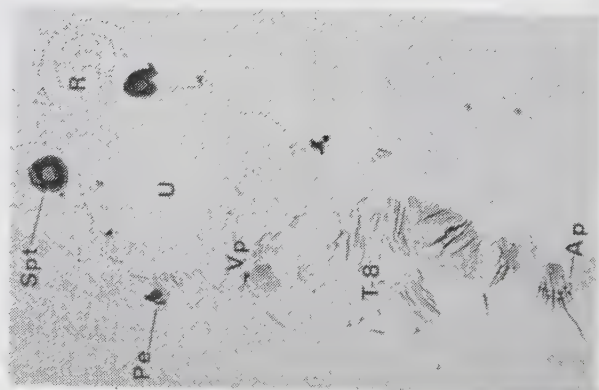
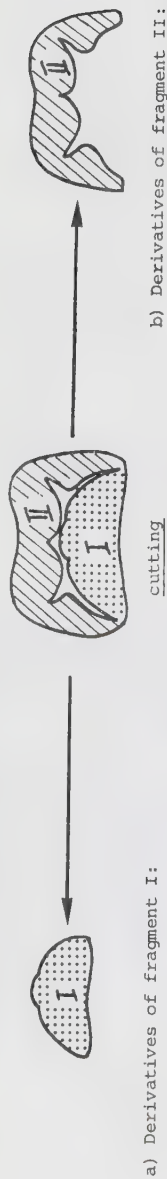
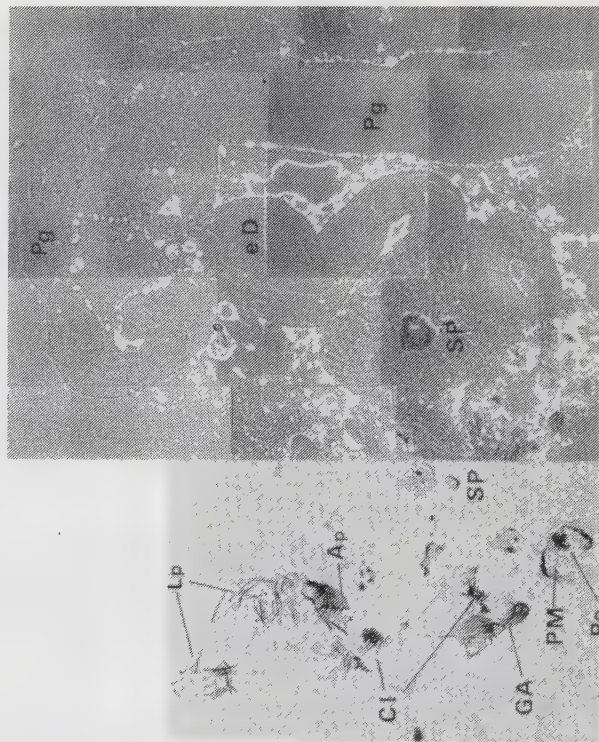
Figure 21 Number of structures produced by fragments I and II of the dsx^D-genital disc:

Figure 22 Localization of the male and female genital primordia in the dsx^D -genital disc:

(Abbreviations: see index of figure 21, p 102)



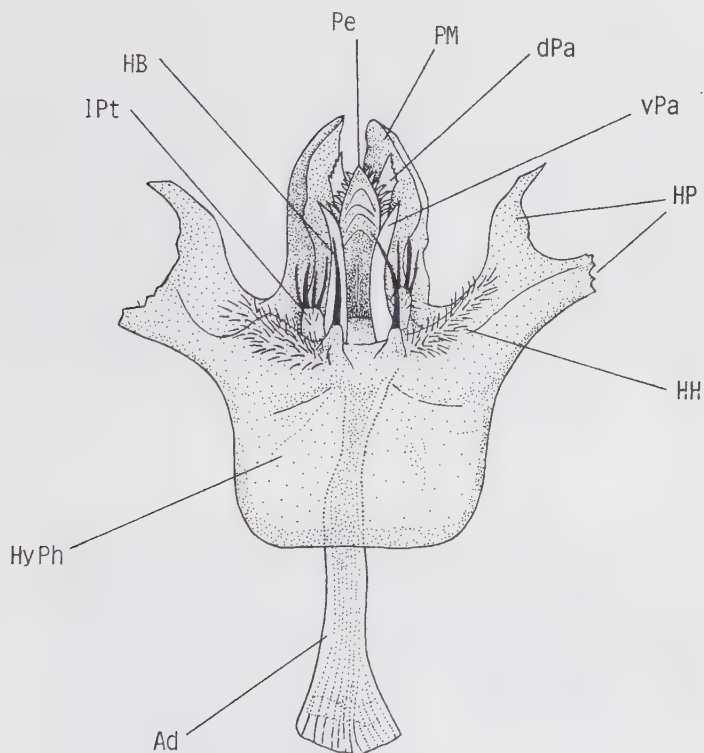
female genital apparatus



external

internal male genital apparatus

Figure 23 Morphology of the penis apparatus:



Legend:

- Pe: penis
- PM: penis mantle
- dPa: dorsal paramere
- vPa: ventral paramere
- HB: hypandrial bristle
- lPt: lateral protuberance with 4 small hypandrial bristles
- HH: hypandrium with hypandrial hairs
- HP: hypandrial process
- HyPh: hypandrial phragma
- Ad: apodeme

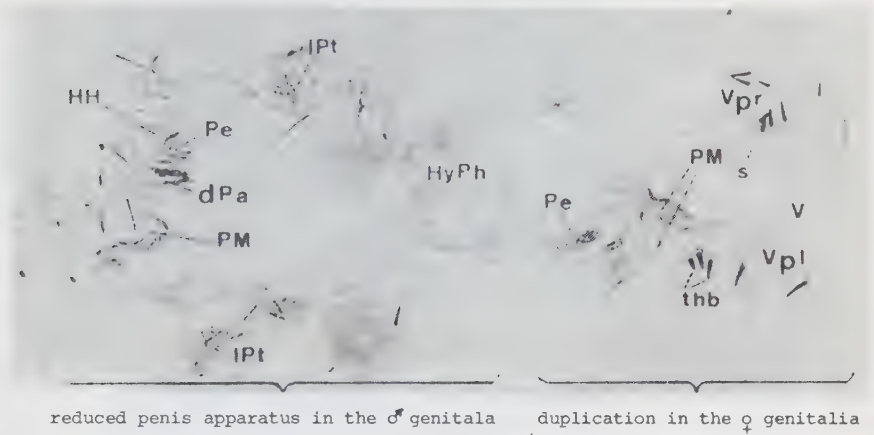
Figure 24

Partial duplication of the penis apparatus in a) XX, dsx/dsx and
 b) XX;dsx^D/+ animals.

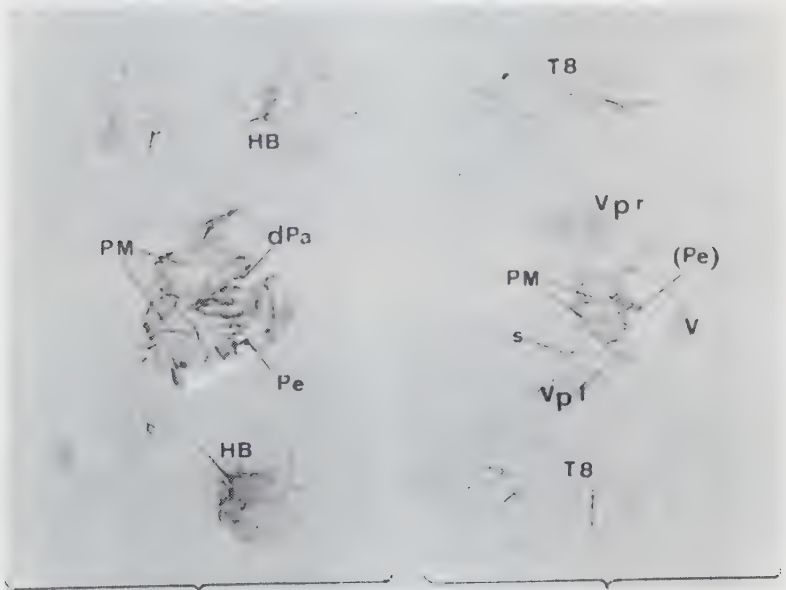
In case where the symbol is written in paranthesis, the identification of the structure was unclear.

Pe:	penis
PM:	penis mantle
dPa:	dorsal paramere
HB:	hypandrial bristle
HH:	hypandrial hairs
lPt:	lateral protuberance
Vpl/r:	left/right vaginal plate
thb:	thorn bristle
s:	sensilla trichodea
V:	vulva
HyPh:	hypandrial phragma
T-8:	tergite-8

Figure 74 Partial duplication of the penis apparatus in the terminalia of intersexual flies:

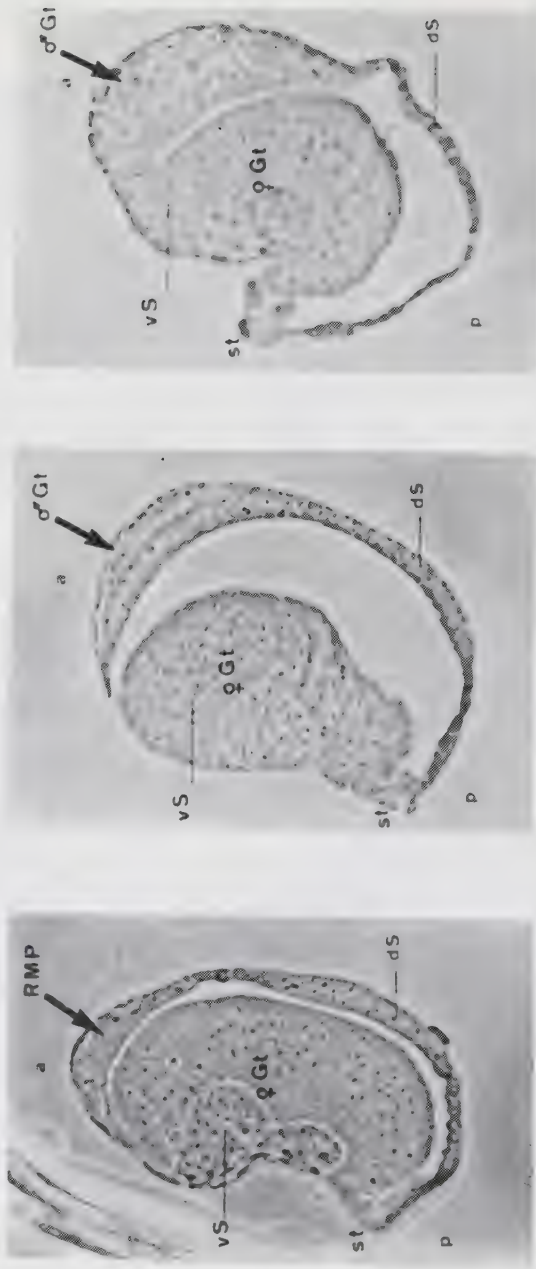


a) XX; dsx/dsx animal



b) XX; dsx^D/+ animal

Figure 25 Comparison of the male genital primordium in sagittal sections of the genital disc of different genotypes (for abbreviations see Figures 5 and 6):



a) wildtype female disc

b) XX; dsx^D/+ disc with reduced male genital primordium

c) characteristic type for XX; dsx^D/+ disc

Figure 26

Developmental alternatives of the terminal primordia:

At blastoderm stage, the terminal anlage consists of separate primordia for the analia (A), the male (M) and the female (F) genitalia.

In the normal female development (♀ pathway), the imaginal cells of the 9th segment fall into the "repressed" state. This "repressed" male genital primordium (RMP) does not form any adult structures.

In the normal male development (♂ pathway), the imaginal cells of the 8th segment form a "repressed" female genital primordium (RFP) which does not produce any adult structures.

The intersexual pathway stands for the mutant condition like in XX; dsx^D/+, XX; ix/ix, XX; dsx/dsx and XY; dsx/dsx animals. There, the male and female genital primordia develop simultaneously. The terminalia of these flies exhibit a male plus a female genital apparatus. The mutant development seems to be somewhat distorted (broken line) and leads to the formation of abnormal terminalia.

Hatched area: male genitalia; dotted area: female genitalia, the analia are marked with stars; dotted line: "repressed" development.

8/9/10/11: anlagen of the imaginal segments.

Figure 26 Developmental alternatives of the terminal primordia

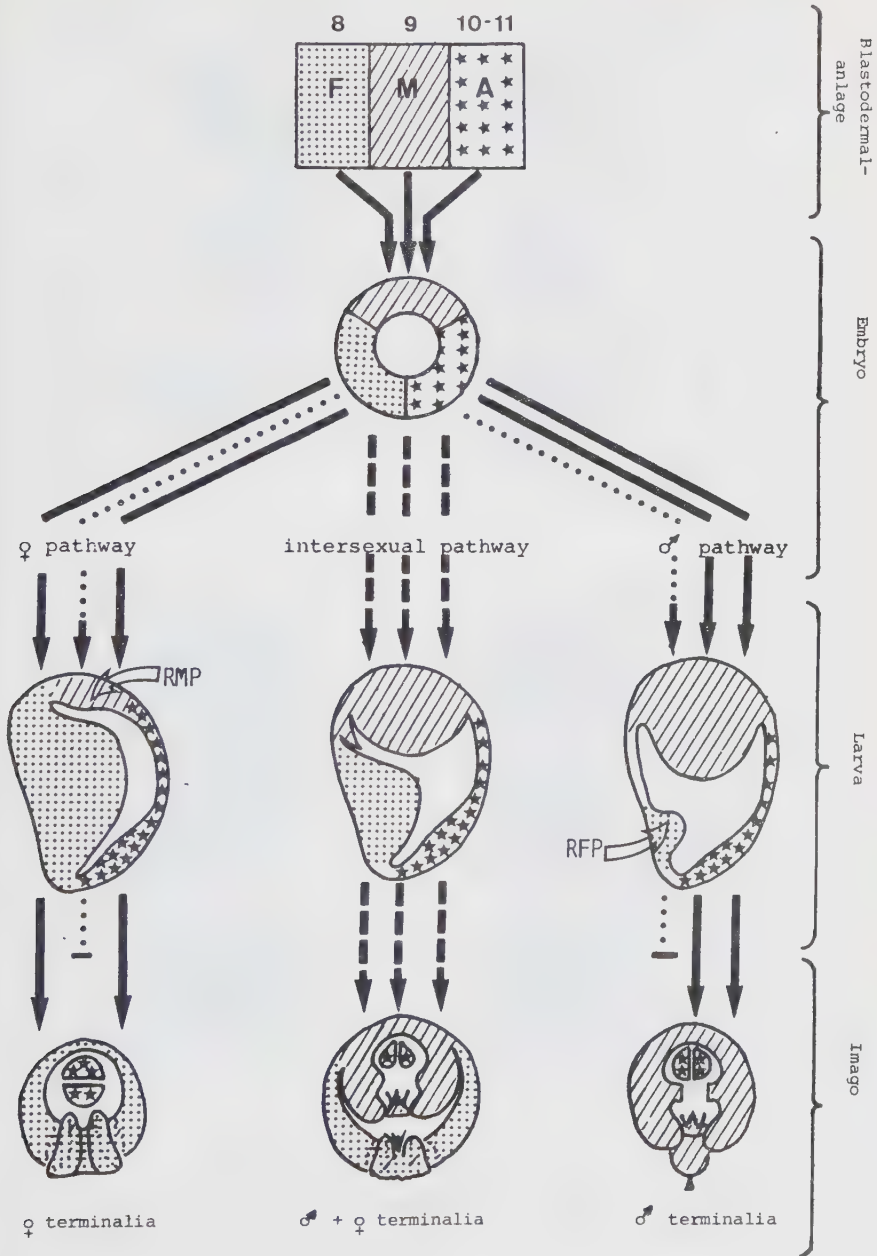
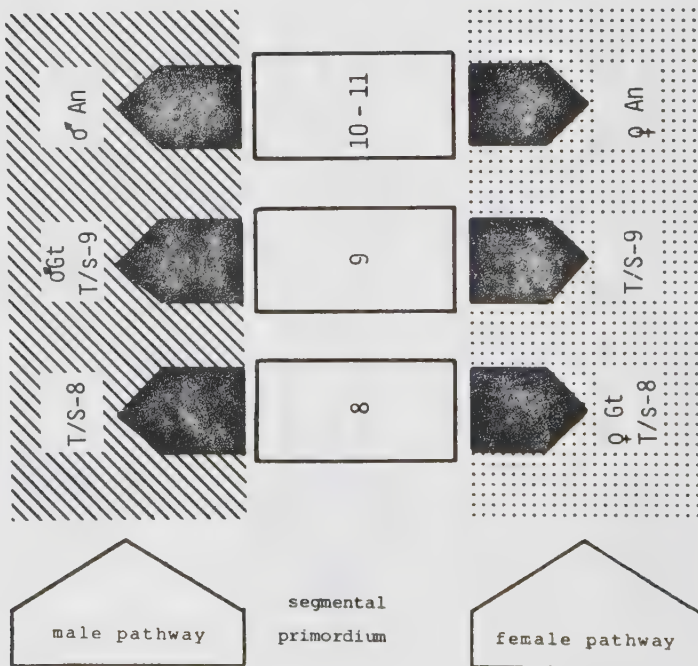


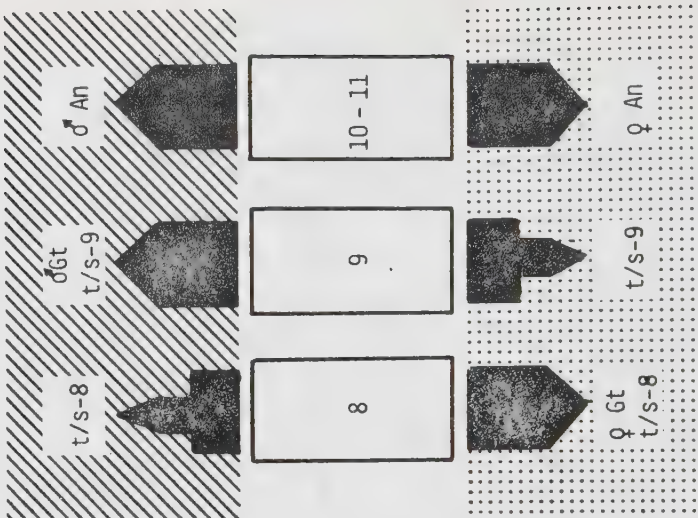
Figure 27

On the evolution of the terminalia (abbreviations see p. 113)

a) primitive Diptera
(hypothetical)



b) first level of reduction
(e.g. Tipulidae)



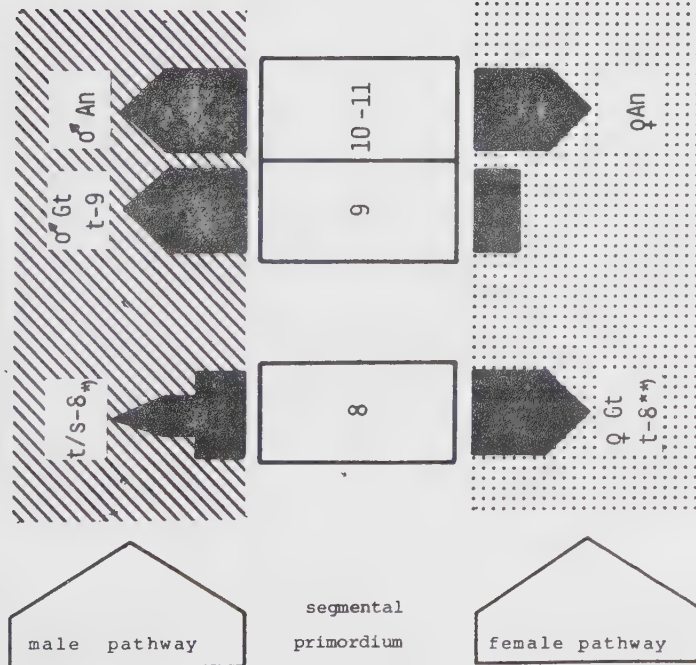
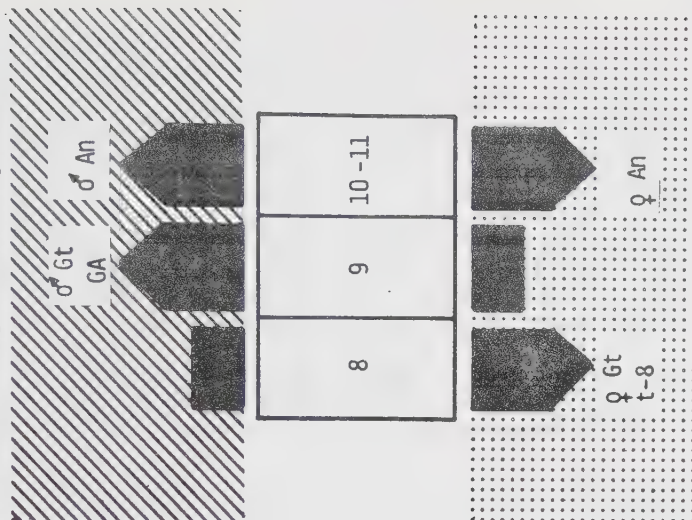
c) second level of reduction(e.g. *Musca domestica*)d) third level of reduction(e.g. *Drosophila melanogaster*)

Figure 27 On the evolution of the terminalia

This figure illustrates two developmental aspects in the evolution of the terminalia. A first one concerns the tendency of the segmental anlagen to fuse into a single genital disc (stage 4). The second deals with the different degrees of reduction of the terminal segments in the male and female developmental programs

Stage a shows a hypothetical situation for primitive Diptera. There, the male and female flies are assumed to have well developed tergites and sternites in segment 8 and 9, besides their genital apparatus.

The reduction of the segments is indicated by a narrowing of the arrow which points to the direction of the alternative male and female pathways.

***) The extreme reduction of segment 8 in Musca domestica is regarded as a specialization of female Calliphoridae and is neglected in the scheme.

*) Males of Musca domestica produce a small tergosternite-8 which is fused to the tergosternite-7.

Abbreviations:

- T: primitive (well developed) tergite
- t: reduced tergite
- S: primitive (well developed) sternite
- s: reduced sternite
- ♂ Gt: male genitalia
- ♀ Gt: female genitalia
- An: analia
- 8 / 9 / 10 / 11: anlagen of the imaginal segments
- GA: genital arch

Figure 28Evagination of the female genital disc

The age of the evaginating disc is given in hours (h) after onset of puparium formation.

Legend:

An : analia

dAp: dorsal anal plate

vAp: ventral anal plate

T : tergite-8

V : vulva

Vp : vaginal plate

U : uterus

R : seminal receptacle

Spt: spermatheca

Pv : parovarium

Od : oviduct

Ov : ovary

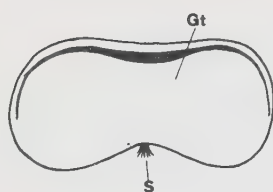
Amp: rectum

S : stalk

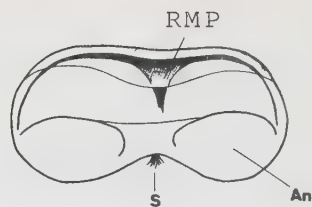
Gt : female genital primordium

RMP: "repressed" male genital primordium

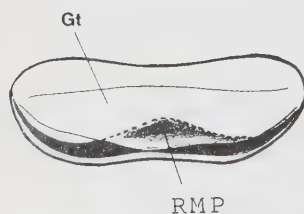
Arrows indicate direction of morphogenetic movements



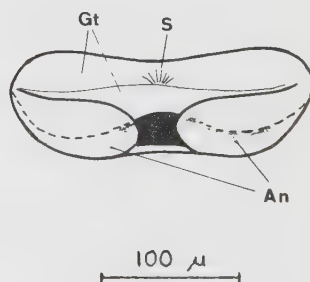
v



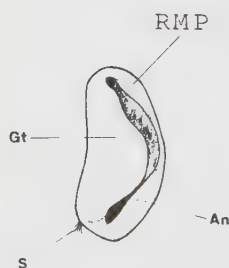
d



a



p



l

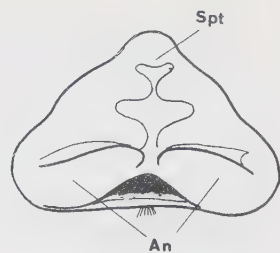
Fig. 28: 0 h
**Evagination of the
female genital disc**

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

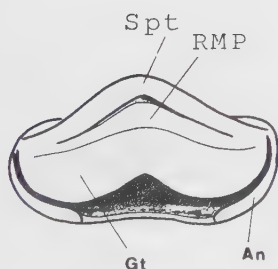
} view



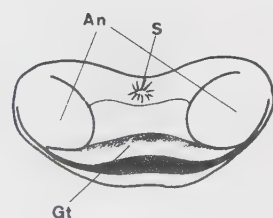
v



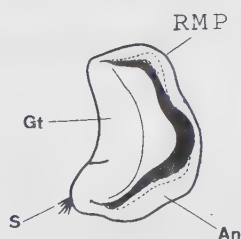
d



a



p



l

Fig. 28: 2 h
**Evagination of the
female genital disc**

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

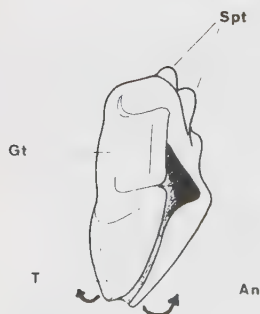
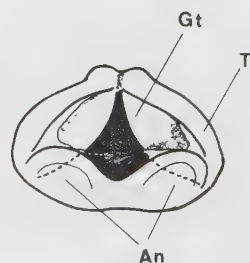
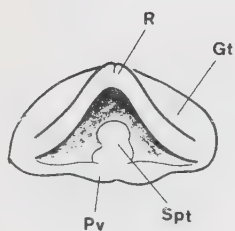
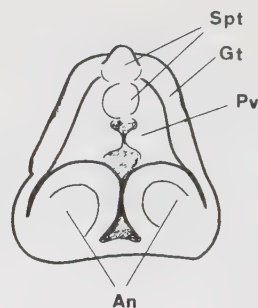
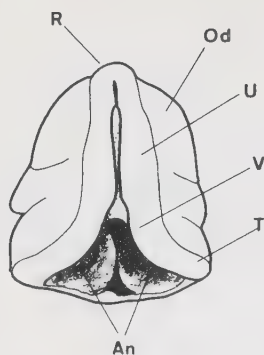
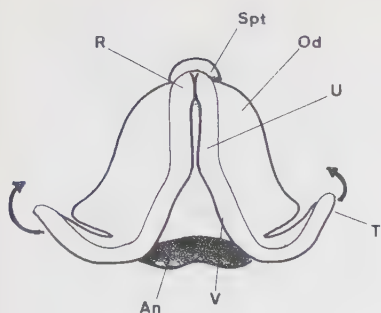


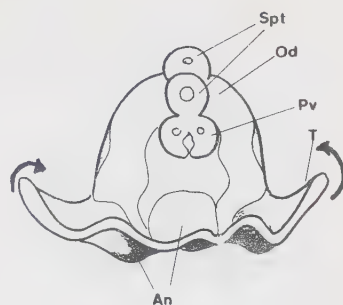
Fig. 28: 6 h
Evagination of the female genital disc

a: anterior
 p: posterior
 d: dorsal
 v: ventral
 l: lateral

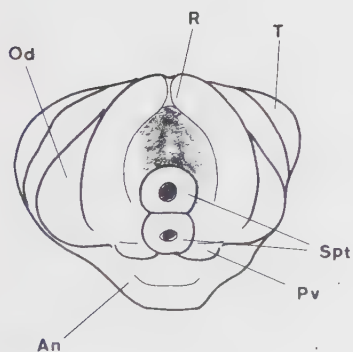
} view



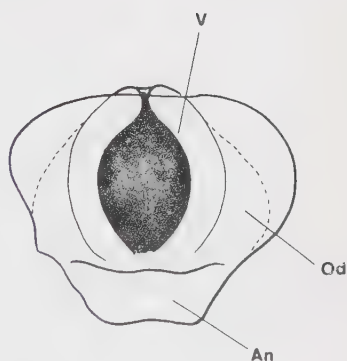
v



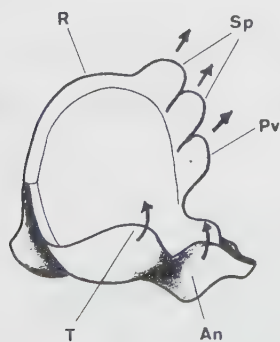
d



a



p

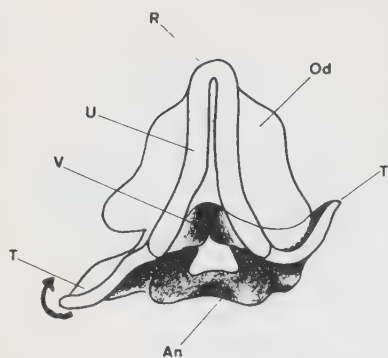


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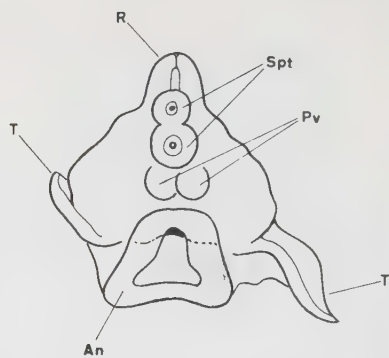
Fig. 28: 12 h
**Evagination of the
female genital disc**

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

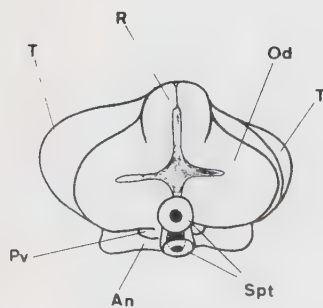
} view



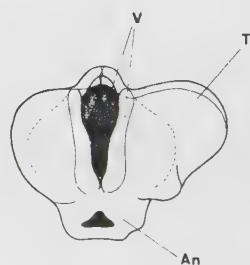
v



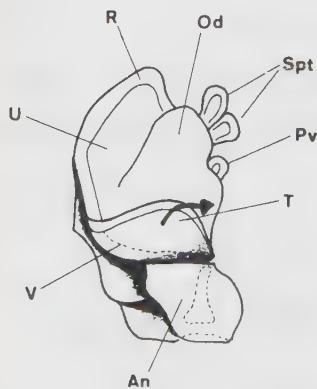
d



a



p

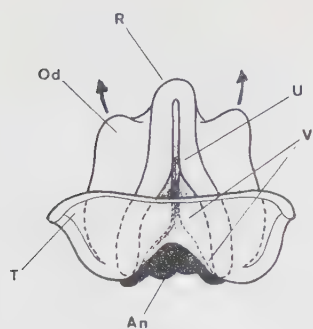


l

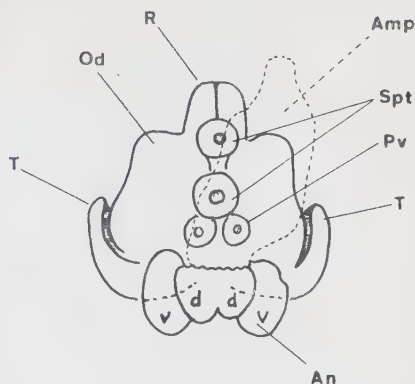
Fig. 28: 16 h
**Evagination of the
female genital disc**

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

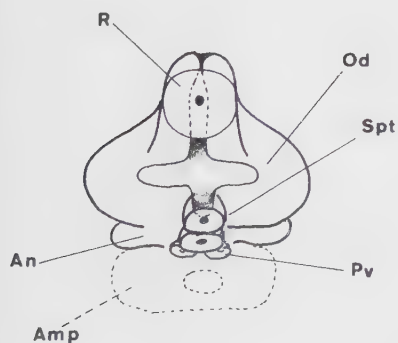
} view



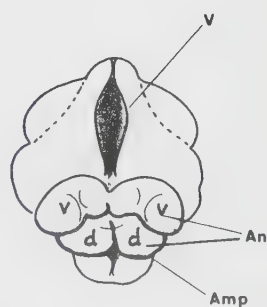
v



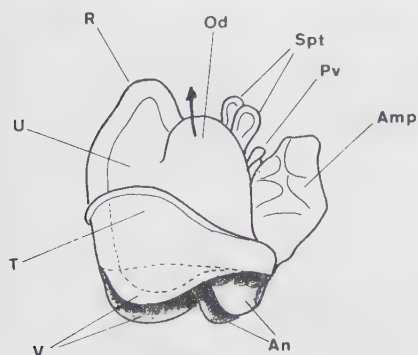
d



a



p



l

Fig. 28: 20 h
**Evagination of the
female genital disc**

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

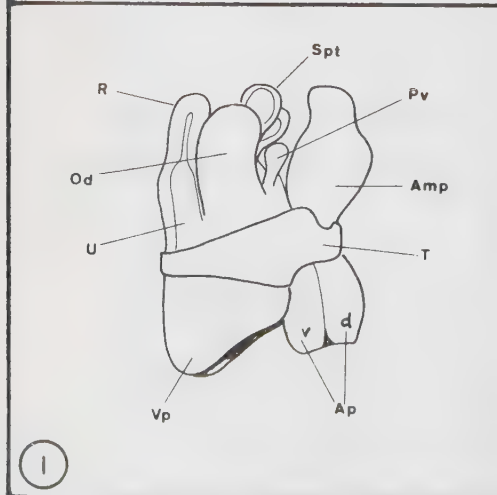
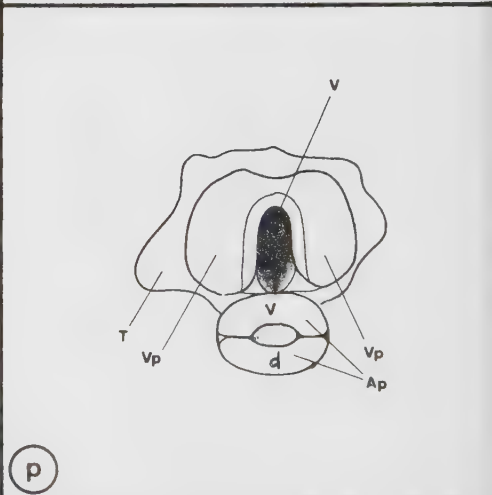
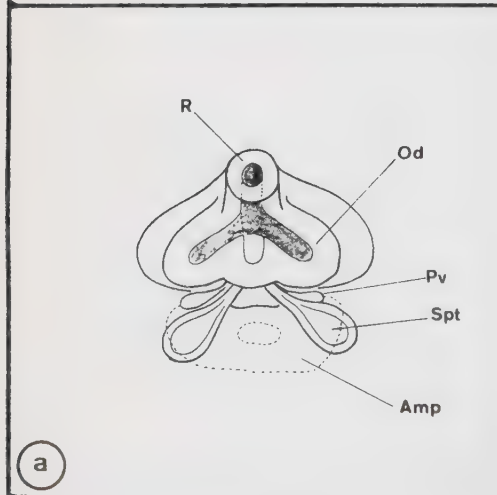
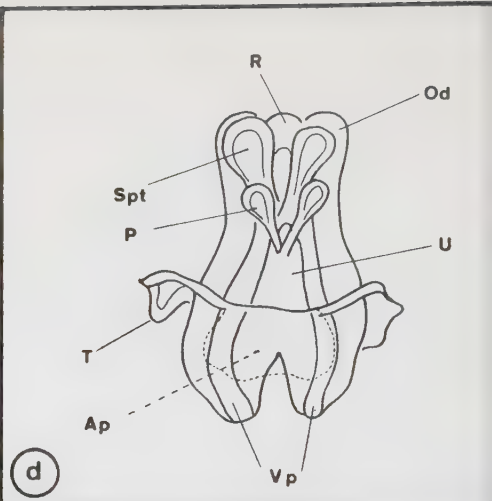
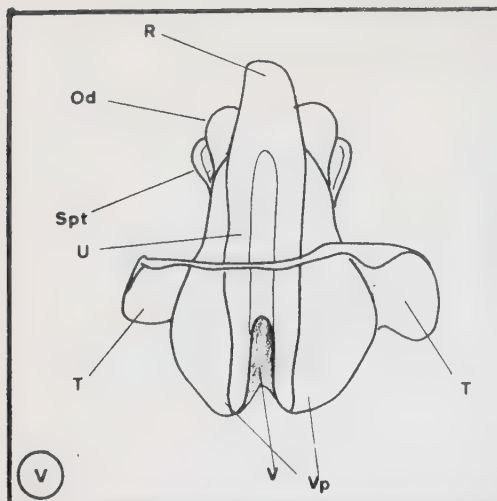


Fig. 28: 24 h
Evagination of the female genital disc

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

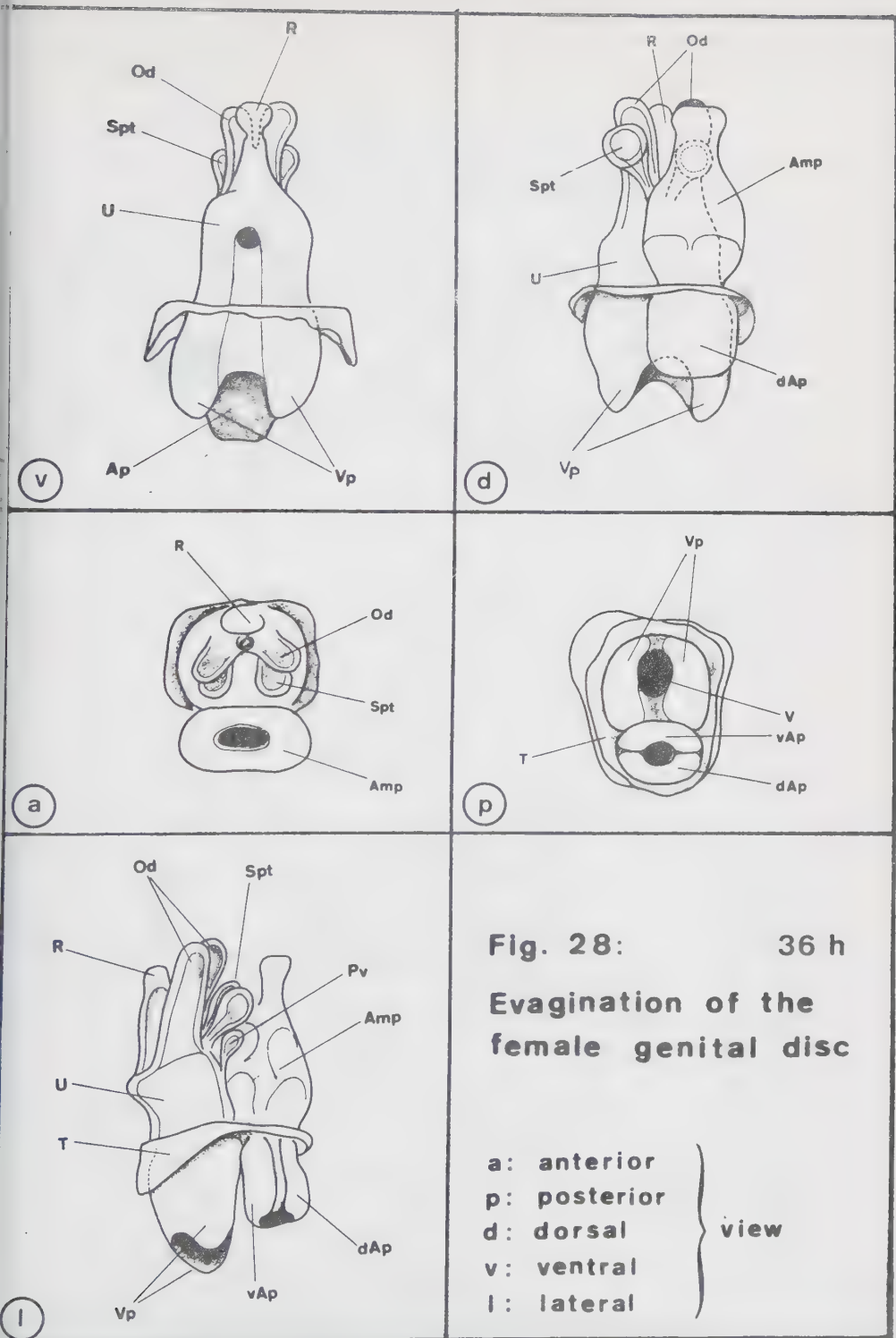


Fig. 28: Evagination of the female genital disc

42 h

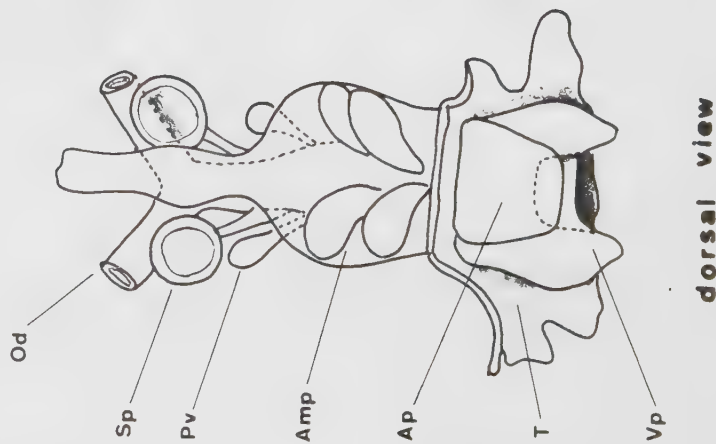
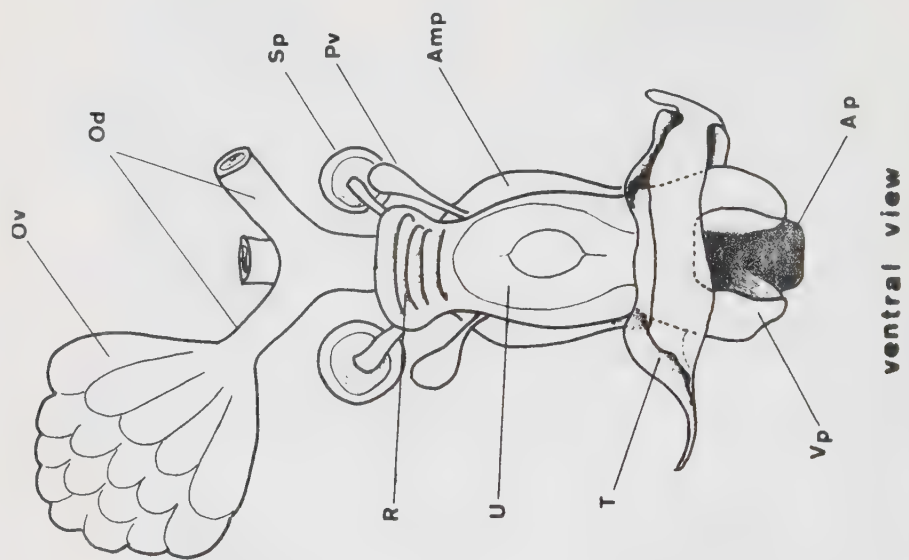
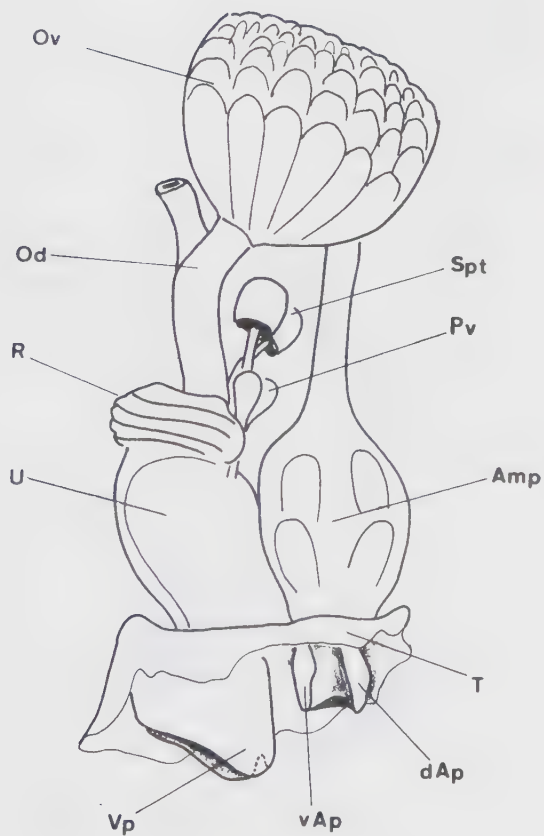


Fig. 28:

42 h

Evagination of the
female genital disc

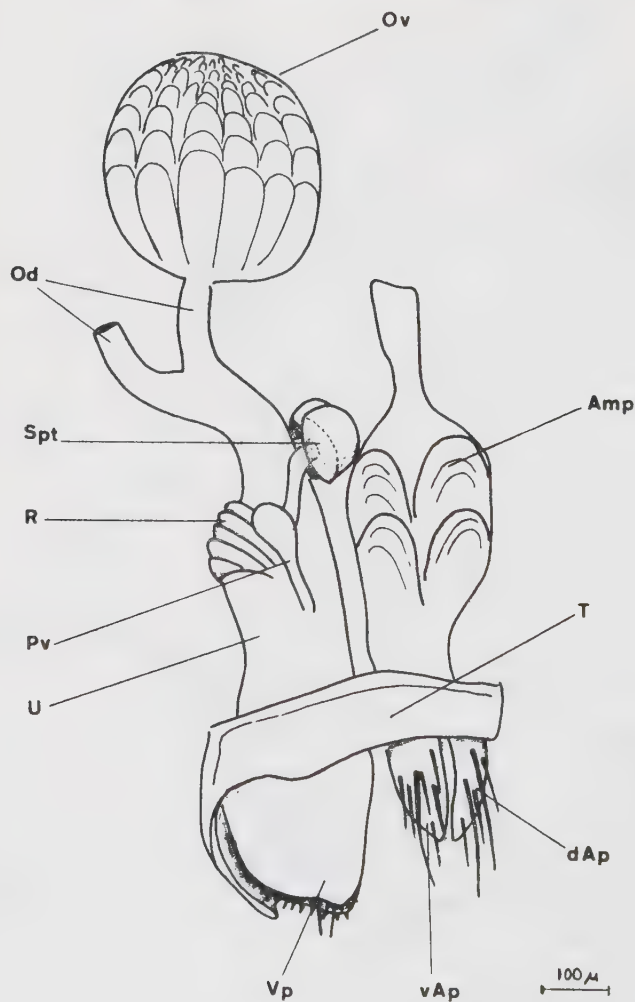


lateral view

Fig. 28:

52 h

Evagination of the female genital disc



lateral view

Figure 29Evagination of the dsx^D-genital disc

The age of the evaginating disc is given in hours (h) after onset of puparium formation.

Legend:

♀Gt: female genitalia	♂Gt: male genitalia
An : analia	Ga : genital arch
Ap : anal plate	L : lateral plate
T : tergite-8	C : clasper
V : vulva	P : penis apparatus
Vp : vaginal plate	SP : sperm pump
U : uterus	D : ejaculatory duct
R : seminal receptacle	Pg : paragonium
Spt: spermatheca	aB : additional bulbus
Od : oviduct	S : stalk
O : ovar	Amp: rectum
	GaL: anlage of genital arch and lateral plate

Arrows indicate direction of morphogenetic movements

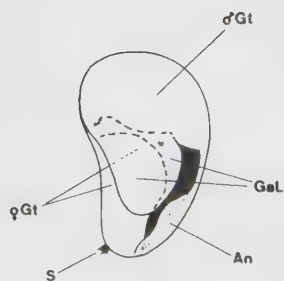
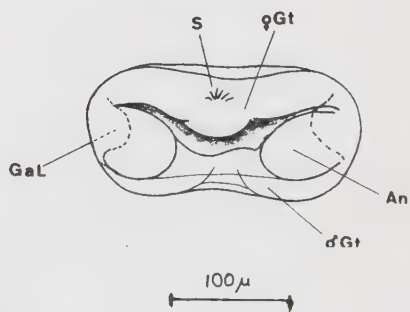
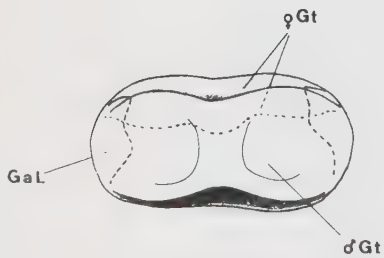
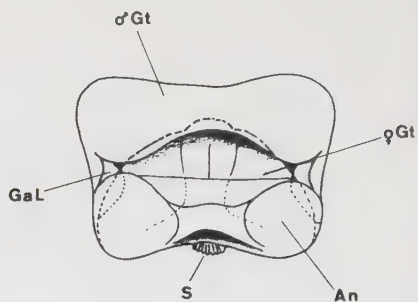
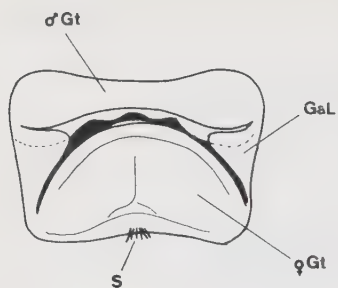


Fig. 29: 0 h

Evagination of the
dsx^D - genital disc

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

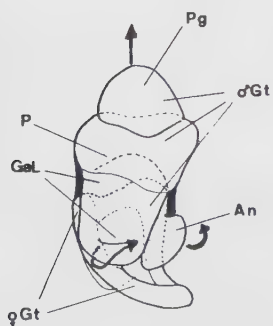
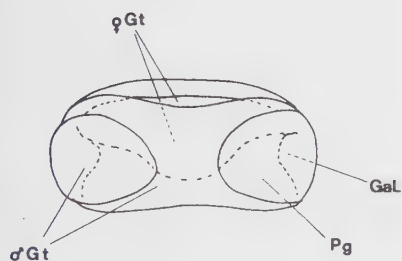
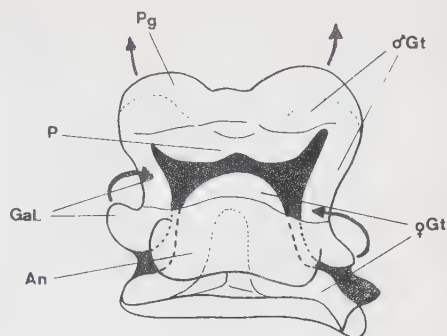
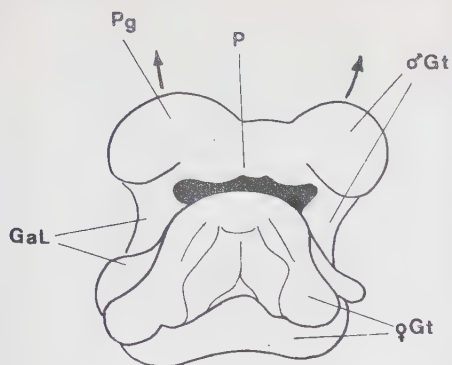
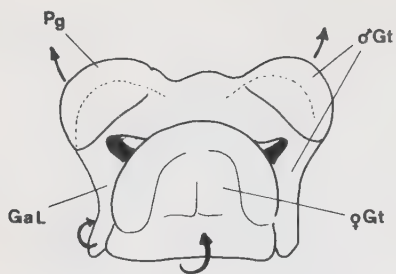


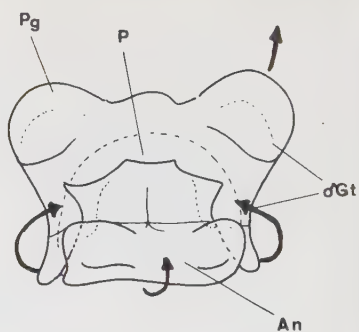
Fig. 29: 3 h
Evagination of the
dsx^D - genital disc

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view



v

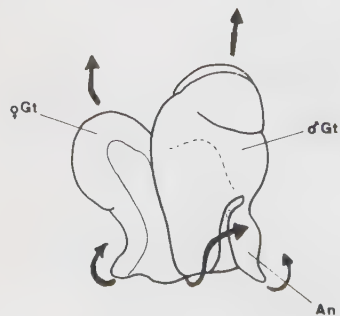


d



a

p



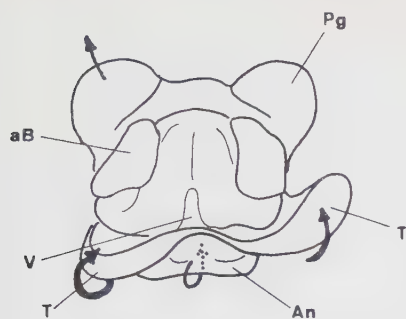
l

Fig. 29: 8 h
Evagination of the
dsx^D - genital disc

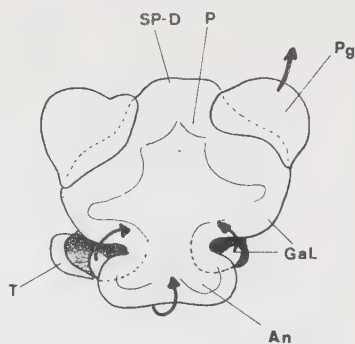
a: anterior
 p: posterior
 d: dorsal
 v: ventral
 l: lateral

} view

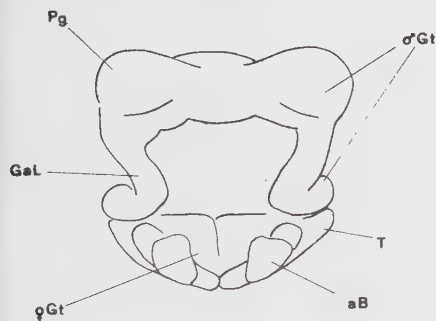
v



d



a

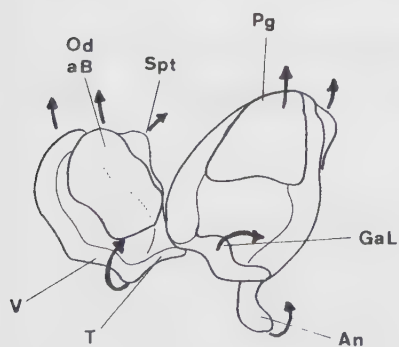


p

Fig. 29:

14 h

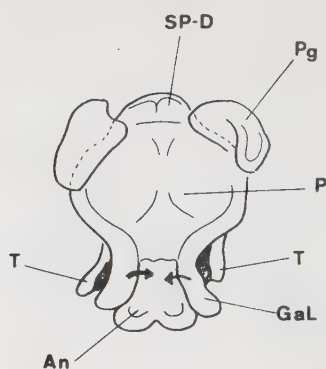
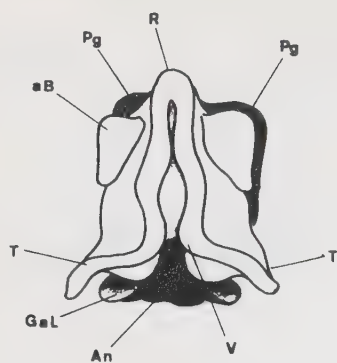
Evagination of the
dsx^D - genital disc



a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

l

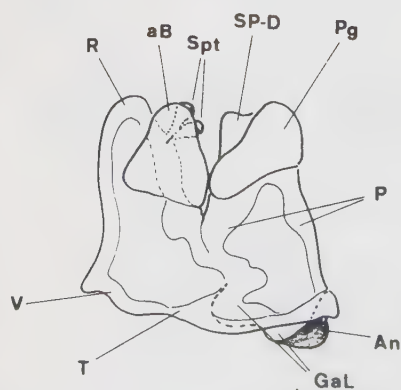


v

d

a

p

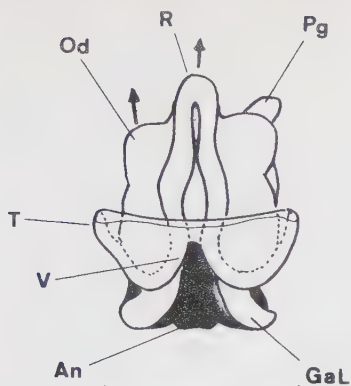


l

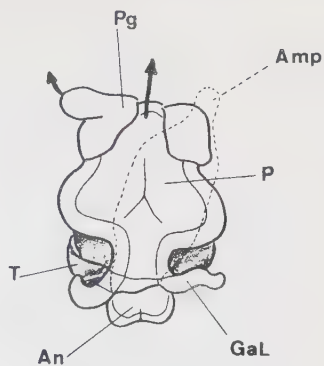
Fig. 29: 18 h
Evagination of the
dsx^D - genital disc

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

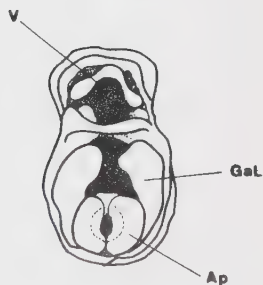
} view



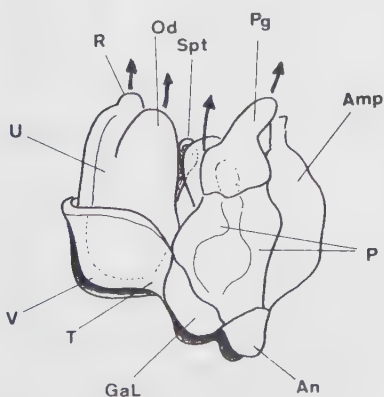
v



d



p



l

Fig. 29: 21 h
Evagination of the
dsx^D - genital disc

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

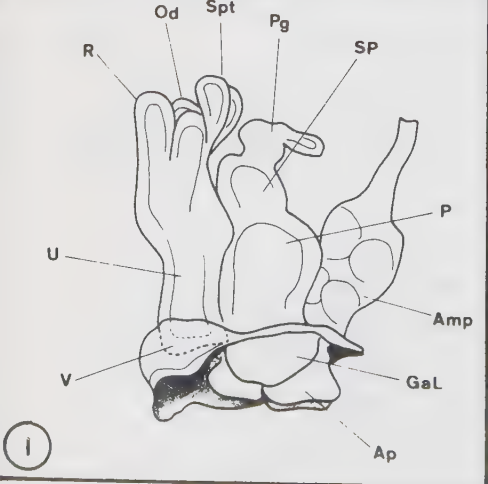
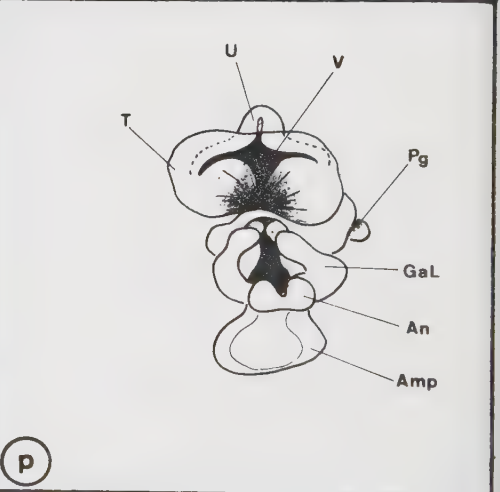
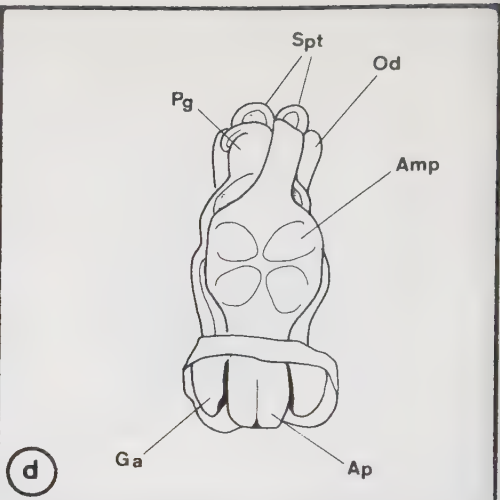
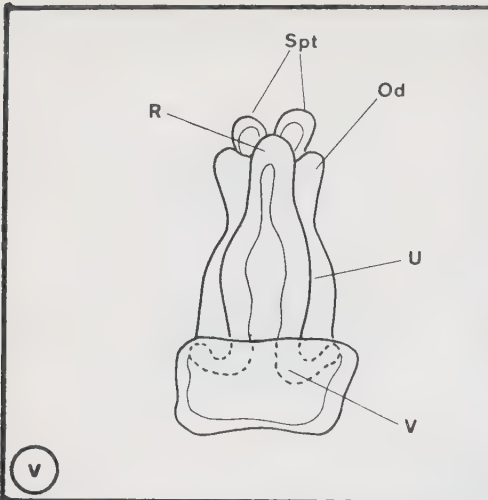


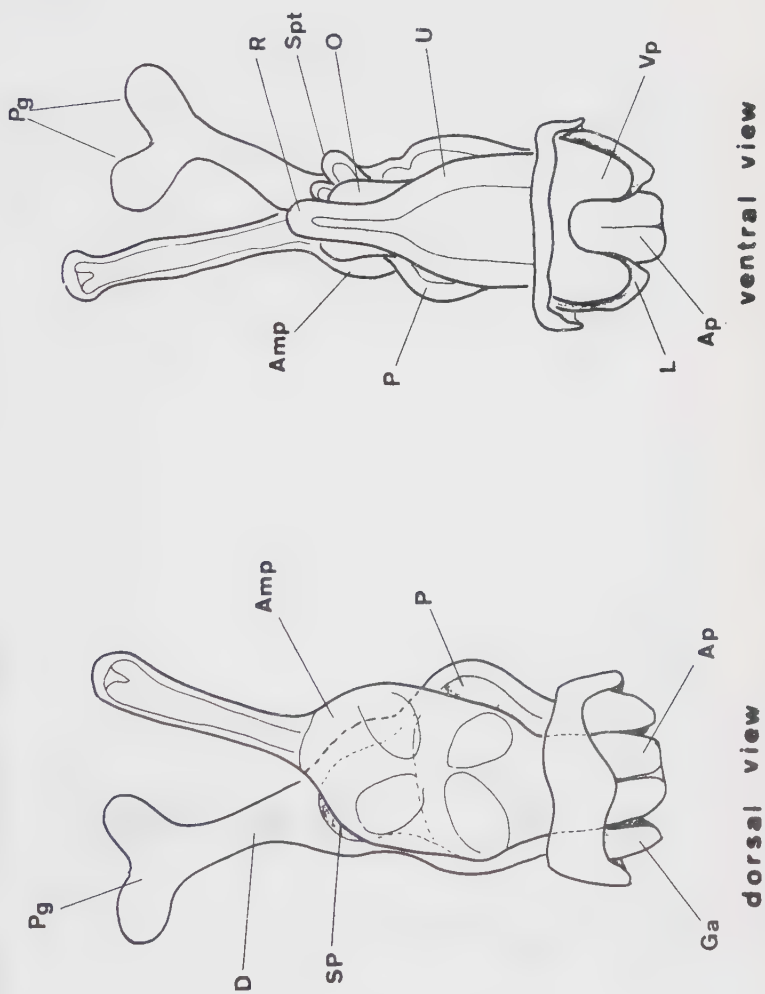
Fig. 29: 24 h
Evagination of the
dsx^D - genital disc

a: anterior
p: posterior
d: dorsal
v: ventral
l: lateral

} view

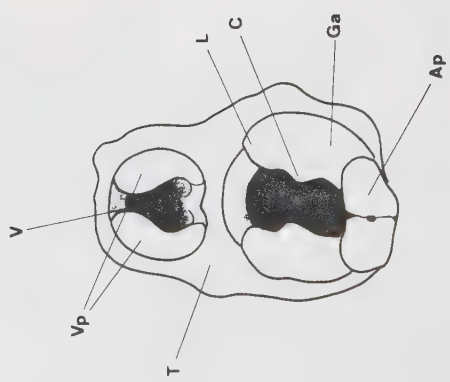
Fig. 29: Evagination of the
dsxD - genital disc

32h

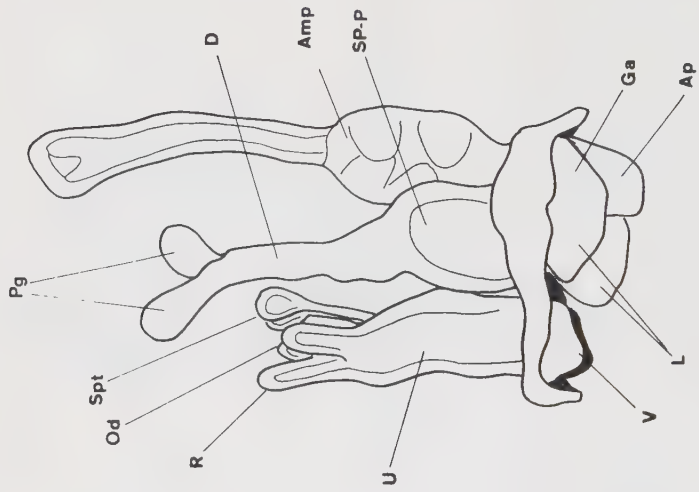


**Fig. 29: Evagination of the
dsxD - genital disc**

32 h



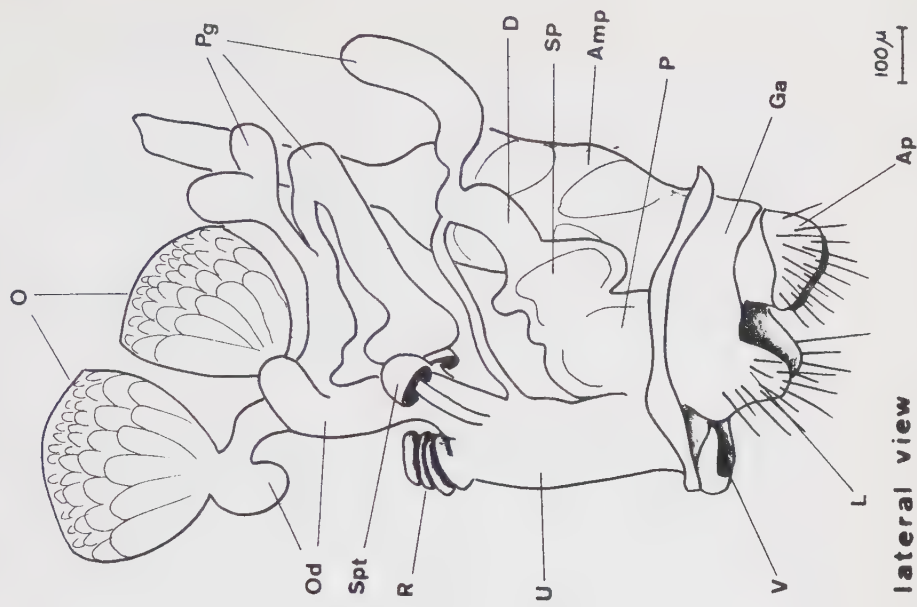
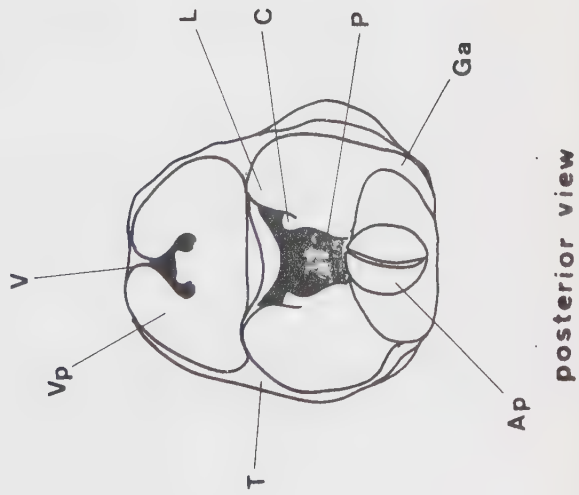
posterior view



lateral view

**Fig. 29: Evagination of the
dsxD - genital disc**

52 h



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Curriculum vitae

Felix Epper-Keller, von Bischofszell und Heldswil (Kanton Thurgau), geboren am 25. Nov. 1948 in Bischofszell. Nach dem Besuch der Primar- und Sekundarschule in Bischofszell trat ich 1965 in das Kantonale Lehrerseminar in Kreuzlingen ein. Während eines Jahrespraktikums unterrichtete ich in Göttighofen (Kanton Thurgau) Schüler der 4., 5. und 6. Primarschulklassen. Nach Erwerb des Primarlehrerpatents im Frühling 1970 unterrichtete ich 1/2 Jahr lang an der Sekundarschule Bischofszell in den Fächern Mathematik, Physik, Zeichnen und Turnen.

Im Herbst 1970 begann ich mein Biologiestudium an der Universität Zürich. Ich besuchte Vorlesungen, Kurse und Kolloquien der Dozenten R. Bachofen, E. Batschelet, J. Biegert, M. Birnstiel, H. Burla, P. S. Chen, C. Claude, C. D. K. Cook, A. Dübendorfer, C. H. Eugster, E. Hadorn, H. Hediger, H.-R. Hohl, E. Jungen-Hauschteck, H. Jungen, S. Kubik, E. Kubli, H. Kummer, R. Nöthiger, H.-R. Oswald, D. Rast, O. Rohweder, J. Schlittler, P. Tardent, B. L. van der Waerden, H. Wanner, R. Wehner, C. Weissmann und V. Ziswiler. Im Herbst 1976 bestand ich das Diplom als Naturwissenschaftler mit Hauptfach Zoologie. Während meiner Diplomarbeit, einer entwicklungs-genetischen Untersuchung des Genitalapparates von *Drosophila melanogaster*, begann ich eine 2 1/2 jährige Tätigkeit als Hilfslehrer in Biologie an der Kantonsschule Freudenberg in Zürich. Seit 1976 bin ich Assistent am Zoologischen Institut der Universität Zürich. Von 1977 an arbeitete ich unter der Leitung von Prof. Dr. R. Nöthiger an der vorliegenden Dissertation.

